An outline of the neuropathology of transmissible spongiform encephalopathies (prion diseases)

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We review here the basic neuropathology of transmissible spongiform encephalopathies (TSE) or prion diseases. The classic hallmark of TSE neuropathology is a combination (in different proportions in different diseases) of spongiform change, astrocytosis, neuronal loss and amyloid plaques. Immunohistochemically, accumulation of the abnormal isoform of prion protein (PrPSc or PrPd) is regarded as a diagnostic for TSE. We also review the peculiarities of kuru, variant Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker disease.

key words: transmissible spongiform encephalopathies, prion diseases, neuropathology, Creutzfeldt-Jakob disease, Gerstmann, Sträussler-Scheinker disease

INTRODUCTION

The transmissible spongiform encephalopathies (TSE) or prion diseases are a group of fatal neurodegenerative conditions which include kuru [28], Creutzfeldt-Jakob disease (CJD) [32], variant Creutzfeldt-Jakob disease (vCJD) and Gerstmann-Sträussler-Scheinker (GSS) disease [90], together with fatal familial insomnia [87] in man, natural scrapie in sheep, goats and mouflons, transmissible mink encephalopathy in ranch-reared mink [15], chronic wasting disease of mule deer and elk in the USA [72], bovine spongiform encephalopathy (BSE) or “mad cow disease” [114] and its analogues in several exotic species of antelopes and wild felids in zoological gardens and feline spongiform encephalopathy in domestic cats.

These conditions are caused by an incompletely characterised pathogen variously referred to as a “prion”, the currently predominant term, or, previously, as a slow, unconventional or atypical virus or “virino”. Despite wide acceptance for the prion hypothesis, these names reflect different views of the true molecular structure of the pathogen and, by the same token, ignorance of its nature.

Those who prefer to view this pathogen as composed “predominantly or exclusively” of a pathologically folded protein PrPSc (the Sc derived from “scrapie”) or PrPd (the d derived from “disease”) use the term “prion”; hence the term “prion diseases”. The virino hypothesis suggests that the pathogen is a molecular chimera composed of a yet undiscovered nucleic acid and a shell-protein which is host-encoded (perhaps PrPd). The virus hypothesis simply suggests that the pathogen is an unconventional virus which has yet to be identified (see: Manuelidis, this volume). The “unified theory” of Weissmann [113], not unlike the virino theory, suggests that the agent is a molecular chimera of the misfolded protein that confers infectivity and an unidentified oligonucleotide, which specifies the strain characteristics.
**NOMENCLATURE**

The nomenclature of PrP species is complex. PrP\(^\text{c}\) is a normal cellular isoform. PrP\(^\text{Sc}\) (PrP\(^\text{res}\) or PrP\(^\text{Sc}\)) is a pathological misfolded protein. PrP\(^\text{Sc}\) is operationally defined as partially resistant to proteinase K (PK) and insoluble in denaturing detergent. In some diseases, however, the pathological isoform of PrP is not PK-resistant [27]. Some researchers, therefore, prefer to use the neutral term PrP\(^\text{d}\), denoting the misfolded species of PrP which is disease-associated whether PK-resistant or not. PrP 27–30 is a proteolytic cleavage product of PrP\(^\text{d}\) sometimes referred to as PrP\(^\text{res}\) (the res derived from “resistant”) when generated following incomplete proteolytic digestion in western blotting.

**PRP, ITS GENE AND THE “PRION HYPOTHESIS”**

PrP is a highly conserved sialoglycoprotein encoded by a cellular gene mapped to chromosome 20 in man and chromosome 2 in mouse [96]. The gene (PRNP) is ubiquitous. It has been cloned in numerous mammalian species, including marsupials, and there are analogues of this gene in birds, reptiles, amphibians, and fish; those in Drosophila and nematodes appear to be cloning artefacts. Pr P27–30 was initially identified as a protein which co-purified with infectivity in rodent brain extracts infected with the 263K strain of the scrapie agent, leading to the conclusion that PrP is part of infectivity.

The “prion hypothesis” was formulated by Stanley B. Prusiner in 1982 [99]. This postulated that the scrapie agent was a proteinaceous infectious particle, since infectivity was dependent on protein, but resistant to methods known to inactivate nucleic acids. A similar proposal had been presented a decade earlier by a number of investigators who had developed earlier suggestion, on the basis of irradiation studies, that the scrapie agent was devoid of disease-specific nucleic acid [74].

Like many amyloid proteins, PrP 27–30 is a proteolytic cleavage product of a precursor protein, PrP 33–35. However, PrP 33–35 is not the primary product of the cellular gene. It has an amino acid sequence and post-translational modifications such as glycosylation and the attachment of a glycosylphosphatidyl inositol anchor (GPI) identical to those of PrP 33–35, but with strikingly different physicochemical features. In particular, PrP is completely degraded by limited proteolysis, which only partially degrades PrP, yielding a core protein (PrP 27–30), which can be visualised ultrastructurally as scrapie-associated fibrils (SAF), better known as prion rods [100]. Prior to conversion to PrP, PrP must be transported to the cell surface, although the site of conversion is uncertain; possibilities include the endosomal-lysosomal pathway, or clathrin-coated pits.

PrP is a glycoprotein with two Asn-glycosylation sites. Thus PrP may exist as deglycosylated, monoglycosylated and di-glycosylated isoforms of different electrophoretic mobilities and glycoforms [17]. The various combinations of the PRNP codon 129 genotype see below and PrP isoform correlate to some degree with the phenotypic expression of human TSE. For example, a distinctive glycosylation pattern with predominance of the diglycosylated species is present in both BSE and vCJD [17, 44]. Although glycosylation patterns “breed true” in that they are retained in experimental passage [17], changes in electrophoretic mobility may occur in the presence of metal ions [17] and more than one isoform may occur in different regions of the same brain, or the brain and peripheral organs, in the same patient. This evidence weighs strongly against the notion that a glycosylation pattern is equal to a TSE strain as defined earlier by Bruce et al. [11].

The PRNP gene in humans consists of two exons, but the whole ORF is confined to the second exon [63]. The polymorphism at codon 129 merits special comment. This encodes Met in approximately 60% and Val in 40% of alleles in the normal Caucasian population. However, in all forms of CJD there is a marked over-representation of homozygotes in relation to heterozygotes. The codon 129 polymorphism may also exert a modifying effect on the phenotypic expression of a given PRNP mutation.

The influence of this polymorphism in kuru is particularly interesting. The practice of cannibalism underlying the kuru epidemic created a selective force on the prion protein genotype. As in CJD, homozygosity at codon 129 (129\(^{\text{Met}}\) 129\(^{\text{Met}}\) or 129\(^{\text{Val}}\) 129\(^{\text{Val}}\)) is overrepresented in kuru. However, Mead et al. [92] found that among Fore women aged over 50 there was a remarkable over-representation of heterozygotes in relation to heterozygotes. The codon 129 polymorphism may also exert a modifying effect on the phenotypic expression of a given PRNP mutation.

**CLASSIFICATION**

For many decades, CJD (the name “Jakob-Creutzfeldt disease” was coined by Spielmeyer in 1922) has been sub-classified into several forms on the basis of clinical and pathological criteria. For instance, Daniel [19] singled out the classical corticostriatospinal (Jakob) type,
of the Heindenhain type (characterised by cortical blindness as a result of the close involvement of the occipital lobes), a diffuse type (dementia with pyramidal and extrapyramidal signs and symptoms) and an ataxic type [10]. Siedler and Malamud [107] discriminated between cortical, corticostriatal, corticostriatocerebellar, corticospinal and corticinigral types. In the literature CJD exists under more than 50 different terms, many of which do not represent CJD in our current understanding of the disease. Discrimination between all these variants is largely of historical interest but recent molecular studies have substantiated the existence of certain defined phenotypes.

PrP<sup>D</sup> (after limited proteolytic digestion) may exist as 21 kDa (type 1) and 19 kDa (type 2) isoforms, which, coupled with the polymorphic status of codon 129 of the PRNP gene, underlie the existence of 7 molecular variants of sporadic CJD (sCJD): MM1, MV1, MM2 — cortical, MM2 — thalamic, MV2, VV1 and VV2. These variants differ both clinically and neuropathologically [14]. Type MM1 corresponds to classical sCJD, with changes in the cerebral cortex, striatum, thalamus and the cerebellum; PrP<sup>D</sup> accumulates mostly as synaptic deposits. This type comprises approximately 70% of sCJD cases. The second most common type, VV2, comprises approximately 15% of all sCJD cases. Changes are confined to the limbic system, striatum, the cerebellum, thalamus and hypothalamus and several brainstem nuclei. The involvement of the cerebral cortex depends on the duration of illness. Those cases of short duration may exhibit minimal cortical change; spongiform change demonstrates laminar distribution while PrP<sup>D</sup> accumulates as plaque-like, perineuronal and synaptic deposits. The MV2 type (approximately 8% of cases) is somewhat reminiscent of the VV2 type in that spongiform change is confined to the subcortical structures. However, in contrast to the VV2 type, the MV2 type shows, particularly in the cerebellum, a predominance of “true” plaques, plaques which are congophilic and visible in a routine haematoxylin and eosin stain. The MM2 type can be further sub-classified into the MM2-thalamic, which corresponds to FFI and FSI cases, and the MM2-cortical, similar to the MM1 type, from which it differs in its limited cerebellum involvement and larger, coarse vacuoles. The VV1 type is very rare (< 1% of all sCJD) and changes are limited to the cerebellar cortex and the striatum, while other structures, including the cerebellum, are barely involved.

A more refined approach was used by Collinge et al. [17, 45], who exploited the size of PrP<sup>D</sup> fragments following limited PK digestion and the relative abundance of monoglycosylated, diglycosylated and deglycosylated glycoforms. This approach discriminated between PrP<sup>D</sup> types 1—4 and type 6; type 5 exists in vCJD-infected transgenic mice but not in humans. All type 1 cases are homozygous for Met at codon 129 of the PRNP gene; type 2 may exist coupled with every status of the PRNP codon 129; type 3 is associated with at least one 129<sup>Met</sup> allele, with the exception of a single CJD case homozygous for Met at codon 129. Type 4, characterised by a predominance of diglycosylated glycoforms, is unique to vCJD and BSE [44]. These types differ neuropathologically as well as clinically. Type 1 cases demonstrated widespread spongiform change in the cerebral cortex, mild changes in the basal ganglia, cerebellum and brainstem but no spongiform degeneration in the hippocampus. In type 2 cases homozygous for 129<sup>Met</sup> the basal ganglia are moderately affected, while in heterozygous type 2 cases or type 2 homozygous for 129<sup>Met</sup> the basal ganglia are closely involved. Type 3 MV cases are characterised by the presence of the kuru plaques already seen in routine haematoxylin and eosin preparation.

The translation of the Collinge scheme into the Gambetti is not straightforward, probably as a result of technical differences in the methodology for PrP<sup>D</sup> analysis by western blot. Furthermore, chelation of metal ions performed prior to PK digestion interconverts both type 1 and 2 MM PrP fragments into so-called 2 PrP [112]. Nevertheless, Collinge’s type 1 MM, type 2 MM, type 3 VV, type 2 MV and type 3 MV are similar to Gambetti’s type MM1, MM2-cortical, VV2, MV1 and MV2, respectively. Thus it seems that the Collinge and Gambetti sub-classifications are basically interconvertible. This notion has been supported by recent work, which indicates that alterations in electrophoretic mobility can be markedly influenced by pH variations in the brain tissue homogenate. When pH is controlled, it appears that two major subgroups of PrP<sup>D</sup> can be identified in terms of the electrophoretic mobility of the unglycosylated band, corresponding to types 1 and 2 of the classification of Gambetti et al.

**CLASSICAL NEUROPATHOLOGY**

In 1920 Creutzfeldt [18] described one case of a novel neurodegenerative condition and Jakob described sequentially 5 cases [50, 51]. Four of Jakob’s cases, still on file at the University of Hamburg, were re-examined by Masters and Gajdusek [89], who confirmed that two of Jakob’s cases fulfil current diagnostic criteria for CJD, while two of the remaining cases represent other poorly-defined neurological conditions. Of special interest is the last of Jakob’s cases, in which there was amyotrophy, initiating the long-lasting confusion surrounding the “amyotrophic type of CJD”,...
which appears to be merely amyotrophic lateral sclerosis with dementia and which is not transmissible [102]. The neuropathological description of Jakob’s cases, which was based on studies of thick colloid section stained according to the Nissl technique, revealed a neurodegenerative process encompassing neuronal loss, central chromatolysis and astroglial proliferation with neuronophagia (Fig. 1, 2). Interestingly, spongiform change was not made visible by the Nissl stain but appeared when the coverslips were removed and sections re-stained with haematoxylin and eosin. The classical triad of CJD neuropathology consists of vacuolation (spongiform change), neuronal degeneration (neuronal loss) and astrocytosis. The changes are bilaterally symmetrical but may be local and, occasionally, even unilateral [116].

**STRUCTURAL CHANGES**

**Spongiform change**

Most characteristic and even “semi-pathognomic” for CJD is the presence of spongiform change (Fig. 3), which remains relatively well preserved even in exhumed cases [110]. Spongiform change consists of small, round or oval vacuoles within neuropil vacuoles that are confluent and form typical “morula-like” aggregates. In the cerebral cortex, spongiform change is confined to the deep cortical layers, the vacuoles in the superficial cortical layers being characteristic of frontotemporal lobar degeneration, including Pick’s disease, or are merely artefactual. It must be stressed that in cases of long clinical duration spongiform change may be masked by the overall loss of neurons, collapse of the cortical cytoarchitecture and robust proliferation of astrocytes. To this end, Masters and Richardson [88] discriminated between “spongiform change” and a “spongiform state” (status spongiosus), the latter consisting of larger cavities of irregular shape in the neuropil (Fig. 4) between a dense meshwork of proliferating astrocytes. Status spongiosus is not specific to TSEs and can occur in the end stage of a wide range of neurodegenerative disorders when widespread neuronal degeneration and loss has occurred. In certain TSE, especially in fatal familial insomnia, vacuolation may be very limited and largely focal, often being confined to the cerebral cortex or thalamic nuclei.

Ultrastructurally, vacuoles are always membrane-bound and contain secondary vacuoles or “chambers” (vacuoles within vacuoles), “curled” membrane fragments and amorphous “fluffy” material of unknown composition. The membranes lining the vacuoles may be simple or multiple [57]. Typical vacuoles originate in neuronal elements, mostly in dendrites or, rarely, axons; those described in astrocytes seems to be fixation artefacts. Spongiform vacuoles have also been studied by scanning electron microscopy (SEM) [16], which revealed ulcerations and defective membranes as well as “rough elevated areas” corresponding to “amorphous membranes” as seen by TEM. SEM has also detected small blisters that are equivalent to small vesicles by TEM.
Vacuoles of the second type originate within the myelin sheath [80]. These are largely non-specific findings but they may be robust in the panencephalopathic type of CJD, in which white matter is predominantly affected and its degeneration does not result from Wallerian degeneration. The diameters of these intramyelin vacuoles are several times greater than those of average myelin fibres and appear “empty”. Within distended myelin sheaths, shrunken axons are observed but many bullous swellings contain no axons. Some axons look normal but others are filled with neurofilaments and scanty electron-dense bodies. Yet other axons are attached to the innermost myelin lamellae by a thin “neck”, probably a mesaxon.

Astrocytosis

Variably severe astrocytosis is observed among almost all neurodegenerative conditions and CJD is no exception [78, 66]. Hypertrophic astrocytes, detected by means of the metal impregnation techniques of Holzer, Kanzler or Cajal (Fig. 5A, B) or, more recently, by immunostaining against glial fibrillary acidic protein (GFAP), are seen in all vacuolated areas. In the cerebral cortex they are particularly prominent in the deeper cortical layers, where swollen or gemistocytic forms are frequently observed. When destruction is so severe that it leads to the collapse of vacuolated neuropil, proliferating astrocytes may virtually replace all other cellular elements. In such a situation the spongiform change may no longer be recognisable. In the cerebellum the proliferation of the astrocytes known as Bergman glia is frequently observed in a wide range of human TSE.

Ultrastructurally, hypertrophic astrocytes are not much different from those from other conditions. They are characterised by abundant glial filaments within the cytoplasm. Liberski et al. [67] have described the close proximity of astrocytes and oligodendroglial cells, but the pathophysiological significance of this phenomenon is uncertain.

There are only two overlapping morphometric studies of astrogliosis in the cerebellum (both Bergmann and velate astrocytes) involving two cases of the ataxic form of CJD [64, 65]. Astrocytes increased from 192.76 ± 117.98 per mm² in controls to 278.08 ± 137.73 per mm² in CJD. An increase in the cross-sectioned nuclear area of Bergmann glia (from 32.72 ± 6.8 μm² to 42.75 ± 9.61 μm²) and of velate astrocytes (from 34.86 ± 7.29 μm² to 39.37 ± 7.10 μm²) was seen when control values were com-
pared with those of CJD. It is noteworthy that the basic three-dimensional geometry of the astrocytic scaffold of the cerebellum was maintained despite a severe loss of granule cells. Electron microscopy revealed several subcellular organelles, rare but otherwise typical for reactive astrocytes, single cilia consisting of ciliary shafts, clusters of interchromatin and perichromatin granules, various adhesive plaque junctions and simple and granular nuclear bodies. Of particular interest is the presence of infoldings of the plasma membranes in the perivascular regions of the astrocytic end-feet. These infoldings were covered by an interrupted or continuous electron-dense undercoat of 30–60 nm in diameter. The latter observation is in agreement with the earlier freeze-etching study of Dubois-Dalcq et al. [24], who showed an increased number of astrocyte-specific particles as opposed to their depletion on membranes forming vacuoles.

**Amyloid plaques**

In GSS, vCJD and some murine scrapie models of disease, “classical” amyloid plaques occur frequently (Fig. 6), although these are often absent in human TSE cases [13], BSE and most ovine scrapie. The presence of amyloid (any protein in a β-sheeted conformation [74]) can be detected *in situ* by tinctorial stains for amyloids, including birefringence following staining with Congo red, immunohistochemistry for PrP, or, in brain homogenates, in the form of fibrillar PrP aggregates labelled prion rods [100]. However, in transmission electron microscopy most of the disease-specific PrP identified by immunocytochemistry in TSE-affected brains is not visibly fibrillar [56].

Typically, amyloid plaques consist of a congophilic PrP-immunopositive dense core (Fig. 7) of densely interwoven amyloid fibrils surrounded by different numbers of dystrophic neurites (DN). The amyloid plaque usually exhibits positivity for other tinctorial stains, including Periodic acid-Schiff (PAS), Alcian blue and various silver impregnation techniques. The PrP-plaque is penetrated by astrocytic processes. Microglial cells were detected in plaques of GSS [4] and in murine scrapie plaques [55]. The exact morphology of the PrP-plaque varies. Thus the “kuru” plaque consists of a stellate core with minimal numbers of dystrophic neurites or none at all [41]. Multi-centric plaques consisting of numerous such cores of different sizes and shapes are typical of GSS [39, 69]. In vCJD, the large fibrillary amyloid plaques

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**Figure 5A, B.** Reactive astrocytes impregnated by the Cajal gold sublimate method. Courtesy of Prof. Herbert Budka, Vienna, Austria.
in the cerebral and cerebellar cortex are surrounded by a corona or “halo” of spongiform change, the so-called “florid” plaque [115].

Neuroaxonal dystrophy

Neuroaxonal dystrophy (NAD) is one form of neuronal degeneration [84] which may occur in apparently normal brains but become “pathological” only if found in increased numbers. The ultrastructural correlate of NAD is a dystrophic neurite, a neuronal process (a dendrite or a myelinated axon) filled with abnormal subcellular organelles such as lysosomal electron-dense bodies or masses of neurofilaments (Fig. 8). NAD has been described in CJD [71], GSS [69] and in numerous experimental scrapie models. Dystrophic neurites in both natural and experimental CJD accumulate phosphorylated neurofilament proteins [70].

Apoptosis and autophagy

As in many neurodegenerative diseases, such as Alzheimer’s disease, caused by the accumulation of “toxic” proteins, neurons in TSEs die via programmed cell death (PCD), of which only the apoptotic process is relatively well characterised. Three types of PCD can be distinguished [26].

Figure 6. Classical unicentric kuru plaque in a CJD brain.

Figure 7. PrP-immunoreactive plaque in a kuru brain.

Figure 8. Neurofilament protein immunoreactive neuritis in CJD; in (B) the enlarged portion attached to the Purkinje cell is a “torpedo” [70].
1) The first type (apoptosis) is characterised ultrastructurally by specific alterations including cell shrinkage, condensation of chromatin and, eventually, the formation of so-called “apoptotic bodies”. The latter are actively phagocytosed by macrophages.

2) The second type is characterised by the formation of numerous cytoplasmic autophagic vacuoles that are subsequently fused with lysosomes. The appearance of autophagy is followed by mitochondria dilatation and the expansion of the Golgi apparatus and endoplasmic reticulum. The molecular mechanism is different from that of apoptosis and consists of a complex interplay of numerous proteins including the TOR kinase.

3) The third type is similar to the second type, except for the almost complete lack of involvement of lysosomes. Ultrastructurally, type 3 cell death is characterised by swelling of intracellular organelles, resulting in the formation of large empty spaces within the cytoplasm. It is of interest that TSE are characterised by the formation of “spongiform vacuoles” within neuronal elements [54]. While these have never been linked to type 3 PCD, the ultrastructural resemblance of “large empty spaces” to “spongiform vacuoles” would appear to merit further study.

By means of TUNEL methodology, apoptotic neurons have repeatedly been shown in both naturally occurring and experimentally induced TSE [23, 58, 59], and some investigators believe that at least a proportion of “dark neurons” may represent those cells undergoing apoptosis. The latter cells appear shrunken and of homogeneously dark cytoplasm. Their real significance is uncertain and many researchers have regarded them as merely fixation artefacts. Furthermore, typical autophagic vacuoles have been reported by us [85] and by others [7, 9] in CJD and scrapie-affected rodent brains. Recently, autophagy was found in degenerating synapses in CJD [108].

Autophagy, being an evolutionarily ancient cellular response to intracellular and extracellular noxious stimuli, may precede or coexist with apoptosis and the process may be induced by apoptotic stimuli. Furthermore, the level of autophagy may define the sensitivity of a given neuronal population to apoptotic stimuli, which may underlie the phenomenon of “selective neuronal vulnerability” [22]. Thus autophagy and apoptosis are interconnected.

Cellular autophagy is a physiological degradative process employed, like apoptosis, in embryonic growth and development, cellular remodelling and the biogenesis of some subcellular organelles, namely multi-lamellar bodies. Nascent immature autophagic vacuoles coalesce with lysosomes to form degraded autophagic vacuoles and in apoptosis only excessive or wrongly placed autophagy causes a pathological process. It is of note that autophagy is greatly enhanced in other brain amyloidoses, Alzheimer disease, Parkinson’s disease and Huntington’s disease, where the signal for autophagy is huntingtin. We here extend these observations using a different model of scrapie and CJD [79].

Definition of autophagic vacuoles

Autophagic vacuoles are composed of areas of the cytoplasm sequestrated with single, double or multiple membranes (phagophores) originating from the endoplasmic reticulum. Sequestrated cytoplasm contains ribosomes, small secondary vacuoles, and occasional mitochondria. Some vacuoles present a homogenously dense appearance.

Formation of autophagic vacuoles in TSE experimental models

Both scrapie models used by us (the 263K and 22CH) revealed similar frequencies and ultrastructural features of autophagic vacuoles and will be described together. The various changes were observed simultaneously in different areas of the same sample but the following description is organised according to our interpretation of their chronological evolution. Initially, a part of the neuronal cytoplasm was sequestrated by concentric arrays of double membranes; the enclosed cytoplasm appeared relatively normal except that its density was often increased. In many affected neurons the electron density of the central area drastically increased. The membranes duplicated within the cytoplasm in a labyrinth-like manner and the area sequestrated by these membranes enlarged into a more complex structure consisting of vacuoles, electron-dense areas and areas of cytoplasm of normal appearance connected by convoluted membranes. It should be noted that autophagic vacuoles form not only in the neuronal perikarya but also in the neurites and synapse [108]. We also observed a large area of the cytoplasm transformed into a collection of autophagic vacuoles of different sizes and it seems that the latter may represent the final stage of autophagy, as the number of such cells increased toward the terminal stage of the incubation period. In experimental CJD and GSS autophagy was also seen, albeit at a much lower frequency.

Tubulovesicular structures

The “tubulovesicular structures” (TVS) (also known as “scrapie-associated particles”) are the only structures unique at the level of thin-section electron microscopy for all TSEs so far examined and have been identified in GSS, CJD, many rodent scrapie models, BSE and in nat-
ural scrapie of sheep [33, 53, 68, 71, 82]. TVS were first described in mice infected intracerebrally with scrapie [20]. It is of note that in natural scrapie in sheep TVS appeared as membrane-bound accumulations of round particles measuring 35 nm in diameter. In some of these an electron-dense core could be demonstrated [6]. TVS have been reported in the majority of models of scrapie in rodents studied so far. In humans TVS were found in CJD [71, 80], GSS [68], vCJD and FFI [80].

At low magnification a process containing a TVS is crowded with structures typically of higher electron density than other elements in this field. In cross-section TVS are smaller than synaptic vesicles but larger than microtubules; their profiles are highly variable. The exact topology of TVS is not entirely clear. In most published electron micrographs TVS have appeared as spheres measuring between 20 and 40 nm in diameter. Some investigators have suggested that the tubular “arrays” of TVS result from overlapping spherical profiles, but we have repeatedly shown short tubular forms of TVS and it is, therefore, evident that TVS are pleomorphic structures existing in at least two forms, spheres and short tubules.

Only limited data are available concerning the number and density of TVS through the incubation time of experimental TSE. In hamsters inoculated with the 263K strain of scrapie TVS were initially observed as early as 3 weeks after i.c. inoculation, but their number increased only with the onset of disease at 9 to 10 weeks after inoculation [83]. It is noteworthy that vacuolation and astrocytosis paralleled the increasing intensity of vacuolation and astrocytosis at the same time as the appearance of TVS and the increase in the number of processes containing TVS paralleled the increasing intensity of vacuolation and astrocytosis. TVS were approximately twice as abundant in terminally ill mice following inoculation and thus followed the appearance of TVS. Similar findings were described by Jeffrey and Fraser [53]. In mice infected with the Fujisaki strain of GSS, TVS were first seen 13 weeks after i.c. inoculation and their numbers increased dramatically at 18 weeks after inoculation, when the first signs of clinical disease were noted [83]. In contrast to 263K, the Fujisaki strain of CJD showed vacuolation and astrocytosis at the same time as the appearance of TVS and the increase in the number of processes containing TVS paralleled the increasing intensity of vacuolation and astrocytosis. TVS were approximately twice as abundant in terminally ill mice following intraocular inoculation as in mice following intracerebral inoculation [83]. In contrast, the final intensity of vacuolation and astrocytosis was not dependent on the route of inoculation. In conclusion, it may be observed that TVS appear early in the incubation period, preceding the onset of clinical disease. Furthermore, in scrapie-infected hamsters, TVS preceded the appearance of other neuropathological changes. The approximately 10-fold lower infectivity titre of the Fujisaki strain of CJD compared to the 263K strain may have accounted for the delayed appearance of TVS in experimental CJD. The apparent correlation between the number of neuronal processes containing TVS and infectivity titre may explain why in cell cultures infected with scrapie TVS could not be found and why their number in experimental scrapie in sheep or CJD in humans was so low [33, 71].

The biochemistry of TVS

The chemical composition of TVS is unknown. Earlier studies have reported that ruthenium red enhances the contrast of TVS. These staining properties of TVS may be interpreted as evidence for the presence of glycosyl residues within TVS. To evaluate whether PrP is part of TVS make-up, we employed immunogold electronmicroscopy [75, 76]. In all models TVS-containing processes were readily detected and neither these processes nor the TVS themselves were decorated with gold particles. Even if amyloid plaques were observed in close contact with TVS-containing neuronal processes, the plaques were decorated with gold particles while the processes remained unstained [75, 76]. At higher magnification amyloid fibrils were clearly visible with numerous round or short tubular particles attached to them. At such a magnification these particles were membrane-bound and their diameter was approximately twice that of amyloid fibrils; they were virtually indistinguishable from TVS. TVS located in areas adjacent to plaques in the 87V model and in areas of diffuse PrP immunolabelling in the ME7 model were also unlabelled with anti PrP antisera.

PrP<sup>d</sup> IMMUNOHISTOCHEMISTRY

Immunohistochemistry (ICC) has become the major diagnostic tool for human prion diseases, by allowing the detection of PrP<sup>d</sup> [14] in paraffin-embedded tissue sections, along with its more refined equivalent the histoblot [109] and the “paraffin-embedded blot” (PET) technique (Fig. 9) [103]. The major drawback of ICC is to get rid of PrP<sup>d</sup> as all available antibodies, including the widely used and commercially available 3F4 and 6H4 antibodies, cannot discriminate between PrP<sup>d</sup> and misfolded PrP<sup>i</sup>. To this end different unmasking techniques are used including hydrating or hydrolytic autoclaving, microwaving and formic acid or guanidinium thiocyanate incubation; a combination of several of these pretreatments may give the best results.

Several patterns of PrP<sup>d</sup> expression are revealed by ICC [14]. These include the most difficult to visualise, the synaptic (Fig. 10), perivascular (Fig. 11), perineuronal (Fig. 12) and plaque-like pattern (Fig. 13). In certain familial forms of CJD, including E200K mutation,
stripes of PrP\textsuperscript{d} immunopositivity running perpendicular to the surface of the cerebellar cortex are visible [52]. If amyloid plaques are visualised by routine neuropathology (haematoxylin and eosin, Congo red, PAS or Alcian blue stainings), these are labelled “plaques”; if they are detected only by ICC and are not visible by routine techniques, they are referred to as “plaque-like deposits”. In chronic wasting disease [72] and in some experimental scrapie models [75, 76] subependymal deposits (subependymal plaques) are visible (Fig. 14). These correspond, ultrastructurally, to areas of low electron density containing haphazardly-oriented fibrils that, when stained with anti-PrP Abs, are heavily decorated with PrP-conjugated gold particles.

Recently intraneuronal PrP\textsuperscript{d} deposits (Fig. 15) have been described [61, 62]. These intraneuronal PrP\textsuperscript{d} deposits may exist as a diffuse type in the neuronal perikaryon; the large intracytoplasmic inclusion bodies are somewhat reminiscent of Pick bodies, intracytoplasmic punctuate deposits and somato-synaptic dots. There is an inverse relation between PrP\textsuperscript{d} accumulation in neurons and overall PrP\textsuperscript{d}-immunostaining. Furthermore, cases with robust PrP\textsuperscript{d}-immunostaining and low intranuclear staining exhibit a lower age of onset. It is of note that similar intraneuronal PrP\textsuperscript{d}-immunostaining was observed in the coeliac, superior mesenteric and stellate ganglia of vCJD cases [38].

PrP\textsuperscript{d} is also present in the peripheral nervous system as well as in the lymphoid tissue including the spleen [12]. In CJD, PrP\textsuperscript{d} was detected in the satellite cells and neurons of trigeminal ganglia [37] and in an adaxonal location in the nerve fibres. These deposits were scant and were observed in 1 out of 9 CJD cases and in 1 GSS case [40]. The paucity of peripheral PrP\textsuperscript{d} deposition remains in strong contrast to the widespread
Figure 11. Perivacuolar pattern of PrP\textsuperscript{d} depositions.

Figure 12. Perineuronal pattern of PrP\textsuperscript{d} depositions.

Figure 13. Plaque-like pattern of PrP\textsuperscript{d} depositions.

Figure 14. An electron micrograph showing distended extracellular subependymal space in which PrP\textsuperscript{d}-amyloid fibrils are floating. Scrapie-infected hamster brain. Original magnification, \( \times 4000 \).
striatum some neurons were vacuolated to such a degree that they looked “moth-eaten”. Neuronophagia was observed. A few binucleated neurons were visible and a torpedo formation was noticed in the Purkinje cell layer along with empty baskets that marked the presence of degenerated Purkinje cells [5]. In the medulla neurons of the vestibular nuclei and the lateral cuneatus were frequently affected; the spinal nucleus of the trigeminal nerve and the nuclei of cranial nerves VI and VII and the motor nucleus of cranial nerve VI were affected less frequently, while the nuclei of cranial nerve XII, the dorsal nucleus of cranial nerve X and the nucleus ambiguous were relatively spared. In the cerebral cortex the deeper layers were affected more than the superficial layers and neurons in the hippocampal formation were normal. In the cerebellum the paleocerebellar structure (the vermis and flocculonodular lobe) was most severely affected and spinal cord pathology was most severe in the corticospinal and spinocerebellar tracts. Astroglial and microglial proliferation was widespread; the latter formed rosettes and appeared as rod or amoeboid types or as macrophages (gitter cells). Myelin degradation was observed in 10 out of 12 cases. Interestingly, the significance of the vacuolar changes was not appreciated by Klatzo et al [60], but “small spongy spaces” were noted in 7 out of 13 cases studied by Beck and Daniel [5].

The most striking neuropathological feature of kuru is the presence of numerous amyloid plaques (Fig. 16), described as “spherical bodies with a rim of radiating filaments” and found in 6 out of 12 cases studied by Klatzo et al [60], and in “about three quarters” of the 13 cases of Beck and Daniel [5]; they became known as “kuru plaques”. These measured 20–60 \( \mu m \) in diameter, were round or oval and consisted of a dark-stained core with a delicate radiating periphery surrounded by a pale “halo”. Kuru plaques were most numerous in the granular cell layer of the cerebellum, basal ganglia, thalamus, and cerebral cortex in that order of frequency. It is noteworthy that in GSS plaques are located in both the granular cell and molecular layers, whereas in CJD plaques are confined to the granular cell layer. Kuru plaques are metachromatic and stain with PAS, Alcian blue, and Congo red, and a proportion of them are weakly argentophilic when impregnated according to the Belschowsky or von Braunmühl techniques. Klatzo et al. [60] reported that plaques were most readily visualised by the Holmes silver impregnation method. It is of historical interest that another unique disease reported by Seitelberger [105] as “a peculiar hereditary disease of the central nervous system in a family from lower Austria” (eige-
nartige familiär-hereditäre Krankheit des Zentral-
nervensystems in einer niederoosterreichischen Sippe) was mentioned by Neumann et al. [94], who was thus the first person to suggest a connection between kuru and GSS and kuru.

Recently, a renewed interest in kuru pathology has been provoked by the appearance of a variant form of CJD (vCJD), resulting from infection by the agent of bovine spongiform encephalopathy (BSE), that is also characterised by numerous plaques, including the “florid” plaques – kuru plaques surrounded by the coronae of spongiform vacuoles. Hainfellner et al. [41] analysed by means of modern immunohistochemistry the case of a young male kuru victim (Kupenota) from the South Fore region whose brain tissue had transmitted the disease to chimpanzees, and McLean et al. [91] examined a series of 11 cases of kuru which are still in the archives of the University of Melbourne. In contrast to the classical studies described above, both papers stressed the presence of typical spongiform change in the deep layers (III–V) of the cingulate, occipital, entorhinal and insular cortices, and in the subiculum. Spongiform change was also observed in the putamen and caudate, and some putaminal neurons contained intraneuronal vacuoles. Spongiform change was prominent in the molecular layer of the cerebellum, in peraqueductal grey matter, basal pontis, the central tegmental area and inferior olivary nucleus. The spinal cord showed only minimal spongiform change.

Immunohistochemical studies have revealed that misfolded PrP was present not only in kuru plaques, already demonstrated to be PrP[Sc]-immunoreactive by Piccardo et al. [98], but also in synaptic and perineuronal sites. In the spinal cord the substantia gelatinosa was particularly affected, as in iatrogenic CJD cases following peripheral inoculation [35].

Gerstmann-Sträussler-Scheinker (GSS) disease

Gerstmann-Sträussler-Scheinker disease was described in 1936 (Fig. 17) and was then re-evaluated by Seitelberger [105] and von Braunmühl [111] and transmitted to chimpanzees by Masters et al. [90]. In the classical papers of Seitelberger [105] and Boellaard et al. [8] the similarities between GSS neuropathology and that of kuru were stressed and preconceived the transmission experiments of Masters et al. Figure 16. Kuru plaques in the Kupenota kuru case. Figure 17. Original illustration from the classical paper by Gerstmann, Strausssler and Scheinker Über eine eigenartige hereditar-familiäre Erkrankung des Zentralnervensystems. Zugleich ein Beitrag zur Frage des vorzeitigen lokalen Alterns. Z. Ges. Neurol. Psychiat. 1936; 54: 736–762.
Following the discovery of a mutation (Pro102Leu) in the PRNP gene by Hsiao et al. [46], many new mutations and families have been described [30]. A detailed study of the first GSS family was published by Hainfellner et al. [39].

A detailed description of all the diverse families is beyond the scope of this chapter and these have been reviewed elsewhere [21, 30]. Only a general overview will, therefore, be provided. The hallmark of GSS is multi-centric plaque (Fig. 18). In contrast to CJD, where kuru plaques occur predominantly in the granular and Purkinje cell layers, multi-centric plaques are present in the molecular layer of the cerebellum [30, 39, 77]. As the name “multi-centric” implies, GSS plaques are composed of several cores of different sizes which merge. In contrast to classical kuru plaques, multi-centric plaques are neuritic, in other words surrounded by dystrophic neurites immunolabelled against neurofilament proteins. In several families dystrophic neurites also contain paired helical filaments (PHF) identical to those found in the dystrophic neurites in Alzheimer’s disease [31, 77]. At the level of electron microscopy separate cores are readily discernible but overlap. Multi-centric plaques fulfil the criteria for amyloid-like kuru plaques in that they are congophilic and birefringent under polarised light. In addition, diffuse PrP deposits may be demonstrated by PrP-immunohistochemistry. PrP in diffuse (or amorphous) plaques is not yet fibrillised. Spongiform change is variable in GSS and its presence depends on the presence of 21 kDa PrP fragments. In classical descriptions GSS was regarded as “system degenerations” [105] and indeed several white matter long tracts have been found to degenerate in GSS.

In 1996 Will et al. [115] reported a novel form of human prion disease which is now known as vCJD. Clinically, this disorder presents as a progressive neuropsychiatric syndrome with psychiatric and/or sensory symptoms at disease onset followed by ataxia, other movement disorders (particularly myoclonus), visual abnormalities and cognitive impairment, resulting in a terminal akinetic mutism. Since the original description of vCJD 154 cases have been identified in the UK, 9 in France, 2 in Ireland and 1 each in Canada, Italy, the Netherlands, Saudi Arabia and the USA. In the UK, the rate of new cases of vCJD has declined significantly over the past 12–18 months. Unlike most other forms of prion disease, this disorder predominantly affects young adults with an average age of onset around 28 years and a duration of around 13 months. All patients who have been genotyped are methionine homozygotes at codon 129 in the prion protein gene.

The neuropathology of vCJD is distinct from other human prion diseases and is characterised by multiple florid plaques. Florid plaques (Fig. 19) are fibrillary structures with a dense core encircled by a pale region of radiating fibrils and surrounded by a ring of spongiform change [115]. These plaques can be demonstrated using silver impregnation techniques and the Congo red, PAS and Alcian blue stains [48]. Florid plaques occur in all layers of the cerebral cortex but are most conspicuous at the bases of the gyri in the occipital and cerebellar cortices. They are also numerous in the molecular layer of the cerebellum.

Ultrastructural studies of the florid plaques in vCJD have demonstrated the masses of radiating fibrils at the periphery with abnormal neurites similar to those
seen at the periphery of the Aβ plaques in Alzheimer’s disease [73]. Neurofibrillary tangles and paired helical filaments have not been identified in vCJD and immunocytochemistry for tau gives negative results. Immunoelectron microscopy showed PrP accumulation both in the amyloid fibrils and in some of the abnormal cell membranes surrounding the plaques [25].

Spongiform change is widespread but often patchy within the cerebral cortex, mostly in a microvacuolar pattern or in relation to amyloid plaques. In contrast, confluent spongiform change is always present in the caudate nucleus and putamen, and focal spongiform change is present in most of the thalamic nuclei, the hypothalamus and globus pallidus, but the posterior thalamic nuclei (including the pulvinar) are spared. Mild spongiform change is detected in the periaqueductal grey matter in the midbrain, in the pontine nuclei and in the cerebellar cortex, often associated with amyloid plaques.

Neuronal loss in the cerebral cortex is most severe in the primary visual cortex. Neuronal loss in the basal ganglia is most evident in cases with severe and confluent spongiform change. In the thalamus neuronal loss was most severe in the posterior nuclei, particularly in the pulvinar, which also showed marked astrocytosis [117]. The severe astrocytosis in the posterior thalamus was best visualised by immunocytochemistry for glial fibrillary acidic protein [117]. This technique also demonstrated astrocytosis in relation to areas of severe neuronal loss and, less frequently, around the margins of amyloid plaques in other brain regions. Neuronal loss and astrocytosis were not conspicuous in the pons, medulla and spinal cord, but were variable in the cerebellum, sometimes most severe in the vermis.

**Immunocytochemistry**

The florid plaques in the cerebral and cerebellar cortex give an intense positive reaction on immunocytochemistry for PrP [48] (Fig. 20). Smaller “cluster plaques” are revealed by immunocytochemistry for PrP in all cases. PrP immunocytochemistry also shows a widespread amorphous pericellular deposition of PrP around small neurons in the cerebral and cerebellar cortices. In the basal ganglia there is a predominantly perineuronal and periaxonal pattern of PrP accumulation (Fig. 21). A synaptic pattern of immunoreactivity with occasional plaques is detected in the thalamus. In the brainstem and spinal cord, PrP-positivity is present at all levels in the grey matter, particularly in the substantia gelatinosa. No PrP accumulation was detected in either the leptomeninges or the dura mater.

**Quantitative neuropathology in vCJD**

Quantitative studies on the first cases of vCJD confirmed the initial morphological descriptions and indicated that the measurable histological accumulation of abnormal PrP deposits in the cerebellum was much greater than in sporadic CJD cases [49]. Furthermore, the selective involvement of the posterior thalamus was demonstrated, with levels of astrocytosis far in excess of sporadic CJD cases [49]. Subsequent quantitative studies have demonstrated that in the cerebral cortex the spongiform change is consistently most pronounced in the occipital cortex, but the relationship between the spongiform change and the presence of PrP amyloid plaques varies in different brain regions [1, 2]. Analysis of the spatial patterns of abnormal PrP deposition in vCJD has found no significant differences between different regions of the cerebral cortex [3]. In addition to quantitative histology, there is the prospect of developing textural analysis techniques to investigate the differences in patterns of abnormal PrP deposition [93].
Non-CNS tissues

PrP accumulation is identified in the retina (Fig. 22) and optic nerve [42], spinal dorsal root ganglia and in the trigeminal ganglia (Fig. 23), but peripheral sensory and motor nerves contain no detectable PrP. Immunocytochemistry for PrP in the adrenal gland, thyroid gland, parathyroid gland, skeletal muscle, bladder, testes, pelvic organs (vagina, cervix, uterus, Fallopian tubes and ovaries), heart, lung, liver, kidney, oesophagus, stomach, pancreas, gall bladder, salivary gland and skin is negative [47, 48].

In contrast, PrP accumulation is identified in follicular dendritic cells and macrophages within many germinal centres in the tonsils (Fig. 24) and within germinal centres in the appendix, Peyer’s patches in the ileum, the spleen and the lymph nodes from the cervical, mediastinal, para-aortic and mesenteric regions and the thymus [43, 47, 48].

Neuropathological studies have been important in identifying vCJD as a novel human prion disease [115]. The diagnostic pathological features of vCJD are summarized in Table 1.

Although florid PrP amyloid plaques have been described in cases of iatrogenic CJD following dura mater graft procedures [106], their number and distribution in the brain is more restricted than in vCJD. In addition, the biochemical features of abnormal PrP in the brain in vCJD on western blot examination is relatively uniform [47] (in contrast to sporadic CJD, where multiple PrP isotypes have been identified [97, 101]). These findings reinforce the need for detailed characterisation of human prion diseases by clinical, pathological and biochemical studies, which can be reinforced by experimental transmission to demonstrate infectivity and to undertake strain typing studies. This has been supported by the identification of a recent case of vCJD in a recipient of a blood transfusion from a donor who had died earlier (after donation) from vCJD [86].
tion of the similarities in the transmissible agent in BSE and vCJD. These findings have been backed up by other independent workers, reinforcing the link between these disorders [11, 17, 95, 104]. As BSE has now been identified in many other countries across the world, it is possible that more cases of vCJD will be identified outside the UK. The future for vCJD in the UK remains uncertain, since it is not clear if the other codon 129 subgroups will be susceptible to this disease. If so, increased numbers of cases might occur over an unknown time period, indicating a need for continued surveillance for CJD, at least in the near future.

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