Spongiform change
— an electron microscopic view

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Spongiform change is a hallmark of transmissible spongiform encephalopathies (TSEs) in prion diseases. They are defined as small round or oval empty spaces in the neuropil. When confluent, they merge to form “morula-like” structures. Their neuroanatomical distribution and grading within each of defined neuroanatomical areas underlie the lesion profile method used to discriminate strains in rodents and in cattle. Ultrastructurally, vacuoles develop within neuronal elements. They are membrane-bound and contain secondary vacuoles and “curled” membrane fragments. Separate type of vacuoles are those develop within myelin sheath. Those vacuoles develop through complicated opening at the major-dense and intraperiod lines. The histogenesis of spongiform vacuoles is unclear and the only hypothesis that they develop through the formation of abnormal configuration of plasma membranes (ACPMs) has never been substantiated. We suggested that vacuoles may develop through process of autophagy.

key words: prion diseases, vacuoles, lesion profile, PrP, autophagy

The hallmark of the transmissible spongiform encephalopathies (TSE) is the occurrence of spongiform change (see Introduction) [42] (Fig. 1). These is defined as small round or oval empty spaces in the neuropil. When confluent, vacuoles merge to form “morula-like” structures [36]. Their neuroanatomical distribution and grading within each defined neuroanatomical area underlies the lesion profile method used to discriminate between strains in rodents and in cattle [8, 15, 16]. Such approaches are not possible for strain characterisation either in sheep or in man because of the variability in vacuolation within strains [2]. Spongiform change is usually symmetrical but asymmetrical change have also been reported [7]. Furthermore, vacuolation may be scanty or even undetectable, as in the case of TME transmitted to mink of the Chediak-Higashi genotype [50] or SSBP1 in sheep [2].

ELECTRON MICROSCOPY

When electron microscopy is employed, spongiform change is equivalent to vacuoles. While many of these are intracellular and membrane-bound (Fig. 2–8), some, perhaps the majority, of vacuoles are unbounded and the originating structures cannot be identified. The membranes surrounding the intracellular vacuoles may be single (Fig. 9) or multiple (Fig. 10–11) [22, 23, 34–37]. The presence of a single membrane lining the vacuole may suggest that they originate from cisterns of smooth endoplasmic reticulum [9]. Vacuoles appear “empty”, but amorphous material, probably of a proteinaceous nature, is often seen. Numerous curled membranes are “pulled off” from the inner leaflets of the vacuoles (Fig. 12) and divide their contents into secondary chambers.

Not only were Marin and Vial in 1964 [49] the first to visualise vacuoles in human CJD by electron microscopy, they had already distinguished vacuoles from en-
Figure 1. Light microscopic view of spongiform change in (A) human Creutzfeldt-Jakob disease; (B) superior colliculus of scrapie-affected mouflon; (C) scrapie-affected goat; (D) mink affected with TME; (E) BSE transmitted to a tiger (*Panthera tigris*); (F-G) BSE transmitted to a puma, in (G) a laminar pattern is well visible; (H) BSE transmitted to the greater kudu; (I) BSE transmitted to a bison; (J-K) Feline spongiform encephalopathy in domestic cats; (L) BSE transmitted to a tiger (*Panthera tigris*), a caudate nucleus. Figs (E-L), courtesy of Dr. G.A.H. Wells, Weybridge, UK.
Figure 2. A vacuole in a brain biopsy specimen of a human CJD brain. Note numerous membranes dividing the vacuole into "secondary chambers". 2B, C. Vacuoles in sheep with scrapie. Courtesy of Dr. M. Jeffrey, VLA, Edinburgh, Seo Hand.

Figure 3. Confluent vacuoles in a brain biopsy specimen of a human CJD brain.

Figure 4. A vacuole in a brain biopsy specimen of a human CJD brain.

Figure 5. Low magnification showing several vacuoles in a brain biopsy specimen of a human CJD brain.
largements of extracellular space. It may be that many vacuoles do originate within the extracellular space and are not caused by oedema but that the mechanisms are not well understood. In a few subsequent classical papers vacuoles were ascribed to either astrocytic processes [6, 17, 24, 58, 60, 61] or to neuronal elements
Figure 12. Numerous curled membranes divide a vacuole into secondary chambers.

[54, 55] or to both [4, 5, 11–13]. It seems, however, that intra-astrocytic vacuoles are just fixation artefacts.

To this end, when the experimental transmission to non-human primates was carried out and perfusion-fixed brains became available, the view that vacuoles form within neuronal elements (mostly dendrites) predominated [28–33]. This notion has largely been confirmed in our experimental studies [41], although some authors tend to the opinion that vacuoles may originate from different sources [25–27, 45–47, 48]. As these authors used some obscure experimental models, such as CJD-infected guinea pigs, it is difficult to verify their claims. In conclusion, it seems that vacuoles originate almost exclusively in neuronal elements.

Despite the abundance of vacuoles, their morphogenesis is totally unknown, although the occurrence of vacuoles in TG3 PrP<sup>0/0</sup> models would suggest that extracellular PrP<sup>d</sup> is sufficient. Some 30 years ago Beck et al. [1] suggested that vacuoles originate through the splitting of “abnormal configurations of plasma membranes” (Fig. 13) — multi-layered structures visible at the adjacent membranes of dendrites or, less frequently, between two presynaptic terminals. These observa-

Figure 13. Abnormal configurations of the plasma membranes. (A–C), Courtesy of the late Dr. Elizabeth Beck, London, UK; (D) unpublished work by Liberski P.P., Brown P., and Gajdusek D.C.
tions were criticised by Gray [18], but the problem has never been resolved. An obstacle to working out a hypothesis of vacuole formation is the fact that by electron microscopy only fully formed vacuoles are seen and no transition from submicroscopic organelle to vacuole has ever been observed. On the other hand, we can see vacuolated mitochondria, dilated Golgi, dilated SR and loss of cytoplasmic content appearing as vacuoles. The trouble is that we see all these in controls as well as in scrapie-affected brains and it is, therefore, difficult to distinguish in the latter, even in optimally perfused brains, a lesion that might be a progenitor of a vacuole from an artefact. In an unpublished work with Peter Gibson we have suggested that vacuoles are formed fast but relatively infrequently at the sites of weakened intercellular contacts. Although it is an obvious problem, it remains unresolved.

VACUOLES WITHIN THE AXON AND MYELIN SHEATH

While vacuoles underlying spongiform change are relatively specific for TSE, vacuoles forming within either the myelin sheath (colloquially called “myelinated vacuoles”) or axon itself are not. They are particularly abundant in the panencephalopathic type of CJD and GSS [39, 43–46, 56, 62]. However, even in the sporadic “polioencephalopathic” type of CJD they may readily be found [40]. Vacuoles were several times greater than the diameter of an average myelin fibre and looked “empty”. Within distended myelin sheaths shrunken axons were observed but many swellings apparently 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 (Fig. 14–20).

In order to understand how the vacuole is formed within the myelin sheath it is worth recalling the normal structure of the sheath. The sheath is composed of concentric layers of lamellae, each one about 10–20 nm thick. The lamellae are separated by gaps of about 10–20 nm, which are filled with a protein called myelin basic protein. The lamellae are joined together by bands of intermediate filaments, which are about 10 nm thick. The intermediate filaments are continuous with the inner aspect of the lamellae and are thought to be responsible for the maintenance of the integrity of the sheath.

Figure 14. A vacuole within a myelinated axon. Note that the myelin sheath appears relatively well preserved.

Figure 15. Two “myelinated” vacuoles in a scrapie-affected hamster brain. Note the shrunken axons.

Figure 16. “Myelinated” vacuoles in scrapie-affected hamster.

Figure 17. “Myelinated” vacuoles in scrapie-affected hamster. A shrunken axon attached to the innermost layer of myelin is visible in one of them.
tric spiral layers of membranes derived from the plasma membranes of oligodendrocytes. The spiral ends on the outside of the sheath at the external mesaxons and on the inside of the sheath at the internal mesaxons. At the mesaxons two surfaces of juxtaposed membranes come together to form “intraperiod lines” which, in higher resolution, are further separated into two lines divided by an “intraperiod gap” [52] (Fig. 21). Thus an intraperiod line is a site where myelin may be opened to the intracellular space. The juxtaposed external faces of the plasma myelin form the “major dense lines” and these, in turn, may be opened to the extracellular space. Along the length of the fibre the fusing is incomplete, as opposing surfaces of the oligodendroglial membranes are not sealed with adhesive plaque junction (although this is true only outside the nodal region; at the Ranvier nodes the membranes are sealed). Thus the complex opening at the major dense and intraperiod lines as depicted in Figures 21, 22 is reminiscent of “reverse myelinogenesis”. Interestingly, vacuoles have never been observed at the nodes of Ranvier, which may lend support to our hypothesis as only the extranodal regions of the myelinated fibres may separate readily; the nodal regions, which are cohesively sealed, are probably much less prone to splitting.

The significance of “myelinated” vacuoles is uncertain but they may simply be sequelae to neuronal changes elsewhere in the brain, Wallerian-type degeneration also being widespread. Wallerian degeneration will be caused by neuronal degeneration and the other changes will be sequelae to, for example, a failing nervous system at the terminal stages of disease. The intramyelin vacuoles are part of the ultrastructural pathology of many toxic neuropathies including bromethalin, hexachlorophene, triethyltin or the derivatives of toxic plants of the genera Stypadra or Heliochrysm (for a review see [42]). In these conditions the noxious stimuli affect the oligodendroglial cells, which degenerate. The degeneration of myelin-forming cells leads to degeneration of the myelin itself. Recently an interesting condition characterised by widespread myelin vacuolation, avian vacuolar myelinopathy (AVM), has been reported in birds, Bald eagles (Haliaeetus leucocephalus), American coots (Fulica americana) and red-tailed hawks (Buteo jamaicensis) [10, 59]. In AVM large vacuoles develop by splitting at the intraperiod lines, but there are many other conditions in which there is also intraperiod line splitting. Thus “myelinated” vacuoles with an opening at the intraperiod lines are not TSE-specific.

While oligodendrocytes express both PrP$^\text{c}$ and its mRNA [51], oligodendrocytes do not support scrapie replication, as shown in transgenic mice created with full-length PrP$^\text{c}$ under the control of myelin basic protein (MBP) promoter, which targets the transgene to oligodendrocytes [53]. Furthermore, PrP$^\text{Sc}$ are not present on oligodendrocytes (Jeffrey, personal communication). In comparison to toxic neuropathies, therefore, the forma-
Figure 21A. A scheme illustrating a myelogenesis. First, two flattened tongues of an oligodendrocyte’s processes (yellow) meet; B. Then, one of these slipped under another to wrapped spirally around the axon (C). The inner (cytoplasmic) leaflets of the processes’ membranes fuse to form the intraperiod lines while two external (exposed to the extracellular space) membranes fuse to form the major dense line. E. A scheme to illustrate the putative morphogenesis of the intramyelin vacuoles: the first (in reality we know nothing which space is opened first!) opening takes place at the intraperiod lines which open intracellular space not unlike that for the formation of spongiform change in the neuropil; the second opening opens the extracellular space at the major dense line. As a result, the vacuole is formed with elements of both inner and outer tongues.

VACUOLATED NEURONS
Vacuolated neurons are typical for the animal TSE of scrapie in sheep and goats (Fig. 22) [14, 19–21, 72–75] and BSE [63–67] and chronic wasting disease (CWD) in cervids [38, 68–71], but occasionally they are seen in human diseases as well. They are only rarely a feature of murine TSE. Vacuoles do, however, occur in non-infected sheep, cattle and pigs. Thus we cannot be sure that the pathogenesis of perikaryonal vacuoles is the same as of neuropil vacuoles. In contrast to the delicate spongiform change of the neuropil, vacuoles in the neurons are much larger. They occur predominantly in the nuclei of the brainstem. They have never been studied systematically by electron microscopy, but from our limited experience it seems that they may differ from those previously described. Furthermore, vacuoles occur in non-infected sheep, cattle and pigs.

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