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# Pseudoviral Hollow-cored Vesicles in Multiple Sclerosis Brain

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**Summary.** Novel, superficially 'virus-like' hollow-cored particles 50–60 nm in diameter were found in the perivascular extracellular space of the brain from a patient who died with acute multiple sclerosis (MS). It is concluded that they are not virions but are derived from myelin undergoing vesicular demyelination. This case demonstrates the need for caution in the interpretation of unusual electron microscopic appearances.

**Key words:** Acute multiple sclerosis – Virus-like particles – Demyelination – Electron microscopy

While the main burden of proof in detection of viruses in CNS disease is borne by the established immunofluorescent and tissue culture techniques, ultrastructural studies may also be of value in certain situations. The credibility or otherwise of electron microscopy (EM) as a tool in such studies depends to a considerable extent on the ability of its practitioners to distinguish viral agents from artefactual, pathological, or normally occurring non-viral structures. Nowhere is this more clearly demonstrated than in the extensive literature on 'virus-like particles' in the brain in multiple sclerosis (MS) (Andrews, 1972; Hill, 1973; Mirra and Takei, 1976; Kirk and Hutchinson, 1978).

The most widely known of these supposed viruses are the paramyxovirus-like intranuclear filaments first described by Prineas in 1972. It has since been shown that such inclusions occur apparently non-specifically in a wide range of apparently unrelated diseases and are almost certainly not viral in nature. Indeed, they appear to be derived in some way from endogenous nuclear chromatin (Lampert and Lampert, 1975; Mirra and Takei, 1976; Prineas, 1975). However, reports of virus-like inclusions are not confined to filamentous nucleocapsid-like structures. For example, three of four kinds of hollow-cored vesicles (HCV) previously reported from the brain in MS as 'virus-like' have subsequently been identified as non-viral subcellular structures (Field and Raine, 1964; Narang and Field, 1973; Tanaka et al., 1976). The present paper reports a morphologically distinct type of hollow-cored vesicle, superficially resembling virus, in the brain in a case of acute MS. An awareness of the occurrence of such endogenous particles in the CNS in demyelinating disease and an understanding of their derivation may prove instructive to those seeking to interpret 'unusual' inclusions in human tissues.

## Materials and Methods

Blocks for electron microscopy were taken at early autopsy (4 h after death) both from plaques and from the apparently normal white matter of the cerebral hemispheres of a 24-year-old man (Case D25) who died from bronchopneumonia following a $1^1/_2$-year history of clinically diagnosed acute MS. The diagnosis was confirmed histopathologically (Dr. I. V. Allen). Details of the clinical history and neuropathological findings are given in Kirk (1979) and of the control material examined, in Kirk and Hutchinson (1978). Tissue for EM was fixed in 2.5% glutaraldehyde in 0.1 M s-collidine buffer, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812. Semithin (1 μm) sections stained with toluidine blue were examined by light microscopy and areas were selected for ultrastructural study. Ultrathin (60 nm) sections double stained with uranyl acetate and lead citrate were examined with an AEI EM801 electron microscope.

## Results

In a single section of a single block from a recent subcortical plaque, a clump of hollow-cored circular profiles was seen in a fold of capillary basement membrane (Fig. 1). They presented as an array of circular electron-lucid structures set against a dark background. At high magnification it was apparent that they were spheroidal vesicles and that the dense background was composed of the fused outer layer of their bounding walls or membranes (Fig. 1, inset).

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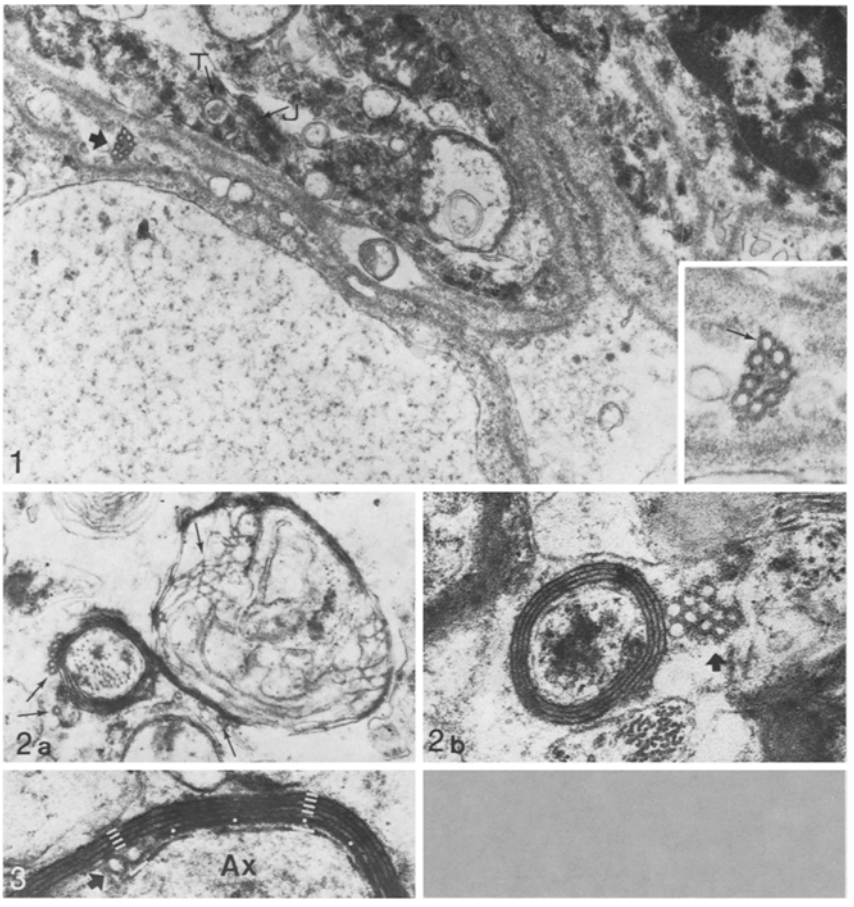


**Fig. 1.** Pericapillary hollow-core vesicles (*arrow*) lying clumped within the brain in a fold of basement membrane. Tight junction (*J*) and tubular body (*T*) identify the adjacent endothelial cell ×18,250. Inset. Detail of hollow-cored vesicles showing the trilamellar wall structure ×46,700

**Fig. 2. a** Adjacent sheaths showing a variety of tubular, vesicular, and net-like appearances (*arrows*) characteristic of vesicular demyelination ×18,250. **b** Clump of vesicles (*arrow*) adjacent to a thinly myelinated axon. ×46,700

**Fig. 3.** Part of transversely sectioned-myelin sheath. On the right the sheath has four distinct major dense lines (*short bars*). Between the innermost of these and the axonal plasma membrane (*long bar*) at least one and possibly two additional major dense lines may be discerned (*dots*). On the left, however, this latter component is absent, being replaced by two tubular-vesicular profiles. Continuity between myelin lamellae and tubule wall is clearly demonstrated. ×46,700

These had a trilamellar substructure formed by two dense granular layers separated by a relatively electron-lucid intermediate layer. The outer dense layer was the thicker of the two but was ill-defined (fuzzy). The overall outer dimensions of these vesicles (discounting the outer fuzziness), ranged from 50 nm to 60 nm. In the same case but in different blocks, a small number of abnormal myelin sheaths was seen. Such sheaths were generally thin and were characterised by the presence of a variety of associated honeycomb, tubular, and vesicular structures (Fig. 2). At high magnification the vesicular structures were seen to have trilamellar walls whose derivation from myelin lamellae was clearly demonstrable (Fig. 3). A fuller description of these appearances, which are interpreted as representing 'vesicular' demyelination, is given in a previous paper (Kirk, 1979). Isolated hollow-cored vesicles remote from myelin and of the kind illustrated in Fig. 1 were unique in our study and have not been seen in any other cases which the author has examined either in MS or in controls. However, they bore a very close resemblance to the vesicles illustrated in Fig. 2, which were seen in association with degenerating myelin in this and other cases (e.g., Kirk, 1979).

## Discussion

Annular structures seen in transmission electron micrographs may represent a variety of three-dimensional structures ranging from spheres to cylinders. For example, there are reports in the CNS pathological literature of small groups of clumped parallel tubules or 'paracrystalline lattices' of unknown significance but mainly found in poorly fixed material (Kaiya et al., 1976; Johnson et al., 1976; Friede, 1978). At low magnification transverse sections of these structures closely resemble the structures seen in the present case. However, closer examination reveals fundamental differences in size (30 – 40 nm as against 50 – 60 nm diameter), in wall structure (single granular as against trilamellar), and in uniformity of apparent lumen size (uniform as against variable to absent). Also, these authors were able to demonstrate longitudinal sections of the tubular components whereas in the present case examination of adjacent sections on either side of that which contained the vesicles revealed no such inclusions. This confirmed the impression that the inclusions were essentially spheroidal rather than cylindrical.

Spheroidal hollow-cored particles of at least four distinct kinds, superficially resembling viruses have been reported previously in MS brain (Field and Raine, 1964; Narang and Field, 1973; Tanaka et al., 1976). It is now apparent that the first of these (Field and Raine, 1964) were the internal vesicles of a normally occurring variety of lysosome, the multivesicular body (Roizin et al., 1967). The second and third particles, both reported by Narang and Field (1973), were subsequently shown to be indistinguishable from nuclear-pore type annuli and altered axonal mitochondria, respectively (Hill, 1973). The fourth and most interesting kind of particle, found in a single case of MS was said to resemble mature intracisternal coronavirus (Tanaka et al., 1976). The particles found in the present case differ morphologically from all those described previously. However, comparison with some of the myelin-related circular structures (Fig. 2) shows many points of close similarity including overall size, lumenal size variation, wall structure, extracellular location, and clumped appearance. It is, therefore, concluded that the most likely interpretation of the perivascular hollow-cored vesicles is that they are derived from myelin undergoing vesicular demyelination.

Although 'virus-like' myelin-derived particles have previously been described in association with degenerating myelin in MS (Suzuki et al., 1969) and in 'Schilder's diffuse sclerosis' (Perier, 1969), in both these reports the particles were unlike the present ones in being indistinct and dense-cored. The extent to which the observed appearances of vesicular dissolution of myelin could result from autolytic or fixation effects has been considered elsewhere (Kirk, 1979) where it was concluded on the basis of the limited published evidence that such effects were unlikely to be of major significance at least within the first 6 h of death.

The appearances of tubular and vesicular myelin-related structure as described herein and more fully in Kirk (1979) are morphologically quite distinct from the tubular-vermiform structures described recently by Prineas and Connell (1978) at the edges of chronically active MS plaques. These latter structures are invariably intracellular the myelin derived tubules being enclosed within membranes originating as invaginations of the phagocytic microglia. The structures described in the present paper, however, seem on the whole to be extracellular. However, it should be readily apparent that if a phagocyte was to endocytose vesicles and tubules derived from vesicular demyelination the resulting structures would be morphologically virtually identical to those resulting from micropinocytosis vermiformis. Indeed, one of the illustrations (Fig. 11) in Prineas and Connell's paper could be interpreted as the endocytotic uptake of tubular myelin. The implication is that some tubular-vesicular disruption of the myelin sheath, occurring extracellularily, preceded the micropinocytosis vermiformis. It is interesting to note, however, that micropinocytosis vermiformis has not yet been described as an accompaniment of vesicular demyelination in animal models of demyelinating diseases[1].

The present findings again point to the need for caution in accepting at face value, viral interpretations for particles found in pathological tissue (Haguenau, 1973; Mirra and Takei, 1976; Yunis et al., 1977). The origins of such particles should first be sought in normal sub-cellular structures with due consideration being given to the possible effects of disease changes, postmortem autolysis, fixation artefact and species differences. Rigorous application of such an approach (e.g., Hill, 1973; Lampert and Lampert, 1975; Kirk and Hutchinson, 1978) has already facilitated the removal of several putative viruses (Prineas, 1972; Narang and Field, 1973; Bauer et al., 1975; Pathak and Webb, 1976) from the list of possible etiological agents in MS.

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1 See note added in proof

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**Note Added in Proof**

While this paper was in press the first report of so called "vermiform demyelination" in an animal model has appeared (Madrid and Wisniewski, 1979). However, unlike Prineas and Connell the authors interpreted the appearances without reference to a micropinocytotic mechanism. Rather they suggested that "some of the vermiform profiles resulted from an abnormal proliferation of oligodendrocyte processes and extensive infolding of their plasma membranes". If this should prove to be the case in MS also, then the distinction between this phenomenon and "tubular-vesicular" demyelination would become even more clear cut.

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