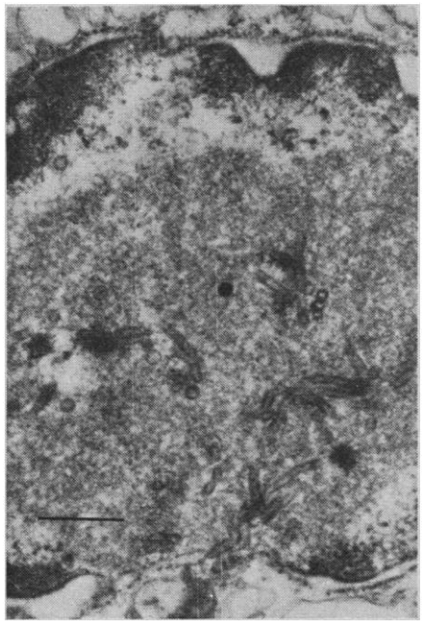

Fig. 3. Electron micrograph of a thin section of an infected oyster, showing tubules within an intranuclear inclusion (bar 0.1 μm, × 85,000).

mine whether the disease was present in the oysters when transplanted to Marsh River, we collected 200 oysters from the Piscataqua River near Eliot on 2 September 1970. Of these, one had pallor of the digestive gland. Light microscopy revealed herpes-type intranuclear inclusions in this specimen, but not in any of 50 other oysters selected randomly from the 200. Electron microscopy (Fig. 2) confirmed the presence of inclusion bodies and virus particles in the single grossly diseased animal. There were no infections in 50 oysters collected from the Marsh River on the same date. However, in 50 oysters collected from the Marsh River in June 1970, there were intranuclear herpes-type inclusions in 5. This is probably the result of a seasonal variation in the prevalence of overt infections. These findings also indicate that the virus infection was present in the Marsh River samples used for the temperature-effect study, and in the Piscataqua River population from which the Marsh River oysters originated.

The evidence suggests that the herpes-type virus infection is enzootic under ambient temperature conditions, and that it was introduced to the Marsh River by importation of infected oysters from the location near Eliot, on the Piscataqua River. The higher mortality of oysters held at higher temperatures correlates well with the high prevalence of herpes-type virus inclusions in that group. The lower mortality among oysters held at lower temperatures likewise correlates with the lower prevalence of inclusion bodies in oysters found where water temperatures were known to be lower. Elevated water temperatures appear to favor spread of the infection or activation of the infection from an occult to an overt phase, or both.

Detailed comparisons of the oyster herpes-type virus infection with herpesvirus in other animals must await further study. However, it is already evident that the virus itself is strikingly similar in fine structure to the Lucké virus, which has been implicated as a cause of renal adenocarcinoma in *Rana pipiens* (*3*). The disease in oysters associated with the herpes-type virus may have a proliferative component, manifest in the cellular aggregates around hemolymph sinuses and in the vesicular tissues. The origin of these aggregates is not clear, but they appear to be derived from hemocytes. It is also not clear whether the cell aggregates represent a self-limited reactive response, or a neoplasm. Herpesvirus infection has been associated with lymphoproliferative disease in monkeys (*4*), fowl (*5*), and man (*6*). Whether there is a relation between the herpes-type virus in the oyster and herpesvirus diseases in other animals, especially man, should be explored.

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Paramyxovirus-Like Particles Associated with Acute Demyelination in Chronic Relapsing Multiple Sclerosis

Abstract. *Electron microscopy of small perivenous demyelinating lesions in a formalin-fixed brain of a multiple sclerosis subject revealed nuclear and cytoplasmic particles resembling paramyxovirus nucleocapsids. These particles, 18 to 20 nanometers in diameter, were found in mononuclear cells related to the central vein and infiltrating the zone of active demyelination. It is suggested that multiple sclerosis lesions may be initiated by seeding of lymphocytes bearing latent paramyxovirus to white matter of the central nervous system.*

Adams and Imagawa reported that titers of antibody against measles are slightly higher in multiple sclerosis (MS) subjects than in healthy subjects of the same age (*1*). Subsequent studies have confirmed this, and also that antibodies against measles are found more frequently in the spinal fluid of MS subjects as compared to healthy subjects and patients with other neurological disorders. The relatively slight elevation in antibody titer and the fact that this is not a constant finding in MS led Brown *et al.* (*2*) to suggest that if measles virus is involved in the pathogenesis of MS, it is present in an unusually masked, latent, or defective form, or that different latent agents may cause the same MS syndrome. Ter Meulen *et al.* recovered an infectious agent related to a group 1 parainfluenza virus from cell cultures established from brain tissue from two MS subjects (*3*). Other efforts to implicate a myxovirus or other virus as an etiologic agent in MS, including electron microscopy of brain tissues, have been unsuccessful (*4*). Acute lesions suitable for electron microscopy have been difficult to obtain, and previous electron microscopic studies in this and in other laboratories have dealt with chronic, subacute, and shadow plaques and white matter remote from plaques (*5*). In the present ultrastructural study of a formalin-fixed MS brain, a sampling procedure with

large sections, 0.5 μm thick, of epoxy-embedded material revealed two early demyelinating lesions suitable for electron microscopy; nuclear and cytoplasmic particles closely resembling paramyxovirus nucleocapsids were prominent in both lesions. Similar material was absent or difficult to detect in subacute and chronic plaques.

The patient (Royal Prince Alfred Hospital No. 186587) was a housewife age 28 at the time of her death. Multiple sclerosis had been diagnosed 5 years earlier following an episode of paresthesia and clumsiness affecting one arm. Routine studies, including a spinal fluid examination, were normal at that time. Three further exacerbations followed by improvement occurred, but from May 1970 her condition steadily worsened, with increasing pyramidal, cerebellar, and sensory deficits; and she died with pneumonia in November 1971. For 3 months starting in January 1971 the patient received azathioprine (150 mg/day); she also received prednisone (5 to 50 mg/day) during the last 3 years of her illness.

Autopsy was carried out 24 hours after death (by J. Fryer), and the brain was placed in buffered formalin. Histological examination confirmed the presence of inhalation bronchopneumonia and typical chronic MS (Fig. 1). Large discrete chronic plaques were present throughout the cerebral white matter, the brain stem, and the spinal cord. Several subacute plaques associated with perivascular cuffs of mononuclear cells and containing numerous fatty macrophages were also seen in the cerebral white matter. Tissue for electron microscopy was obtained from macroscopically normal white matter and from chalky (subacute) and chronic plaques. Slices of tissue 0.5 mm thick and 4 mm square were transferred to cacodylate-buffered 2 percent glutaraldehyde for 48 hours, then placed in Dalton's solution for 2 hours, and subsequently embedded in Spurr's low-viscosity epoxy medium. Horizontal sections (0.5 μm thick) stained with methylene blue were used to select areas for electron microscopy. Frozen sections of the corresponding areas of the formalin-fixed tissues were prepared for routine histology.

Two early demyelinating lesions were found in the 40 blocks examined. Both were located in white matter that appeared normal macroscopically. Each measured less than 0.7 mm in diameter and consisted of a zone of active myelin breakdown disposed around a central vein. One lesion was identified in corresponding frozen sections that revealed the typical appearance of early perivascular demyelination, with preservation of axons in the presence of fatty macrophages and a mild mononuclear cell infiltrate (Fig. 2). No definite inclusion bodies could be identified in these lesions, but in later examinations it was possible to identify in the epoxy sections the type of nuclei which on electron microscopy were found to contain virus-like material; these were large nuclei that stained an even, light blue, with a more darkly staining irregular elongated structure in the center of the nucleus. These cells, which could not be identified in the frozen sections, were present near the central vessel and among nerve fibers undergoing demyelination (Fig. 3).

Electron microscopy of the smaller acute lesion revealed some loss of myelin adjacent to the central vein. Macrophages containing numerous lipid vacuoles were prominent near the vein, in the Virchow-Robin space, and among the remaining myelinated nerve fibers. There was no evidence of gliosis. Intact myelin sheaths revealed autolytic distortion, splitting, and vesiculation; and it was not possible to define the fine structural changes that accompanied demyelination. Groups of lipid vacuoles, usually at some distance from the central vessel, were associated with large cytoplasmic fragments containing aggregates of randomly arranged tubules; individual tubules had an outer diameter of 18 to 20 nm and an inner (core) diameter of 8 to 10 nm, and they were cross-striated with a repeat distance of 6 to 7 nm (Fig. 4). The origin of these cells could not be determined. Nuclei packed with similar but more clearly defined tubules were seen in the Vir-

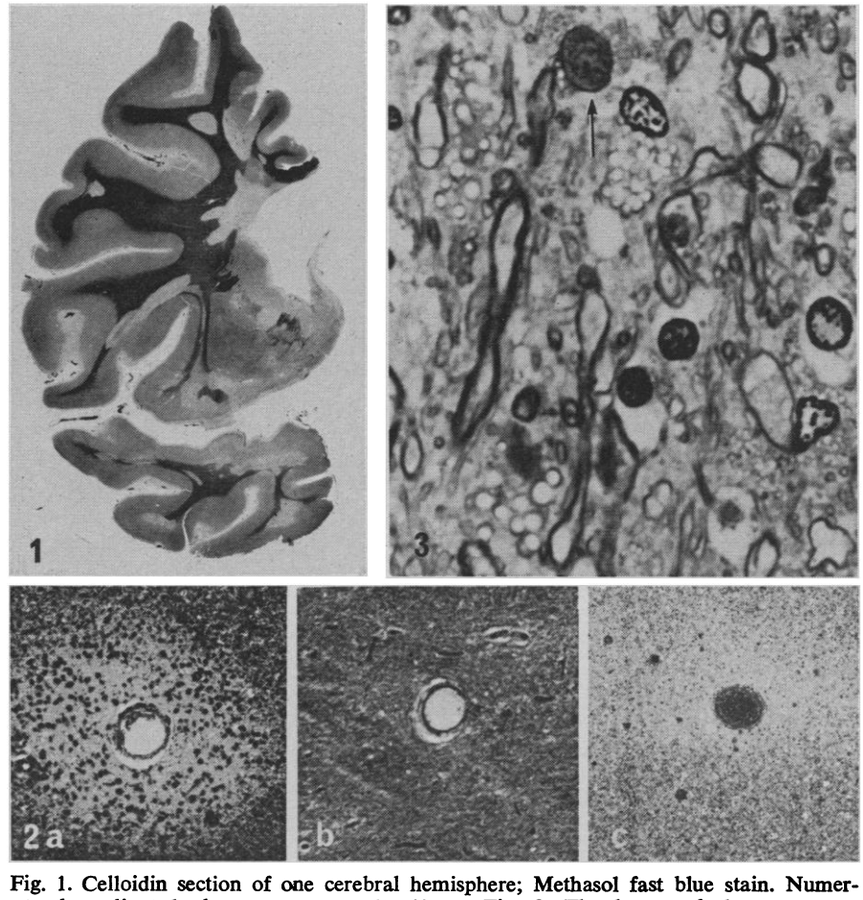

Fig. 1. Celloidin section of one cerebral hemisphere; Methasol fast blue stain. Numerous demyelinated plaques are seen (× 1). Fig. 2. The larger of the two acute perivenous lesions examined by electron microscopy. (a) Fatty macrophages are present in the demyelinated zone around the vein; frozen section stained with hematoxylin and oil red *O*. (b) Holmes silver stain of frozen section after defatting reveals axons preserved in demyelinated zone. (c) Section (0.5 μm thick) of the same lesion has been embedded for electron microscopy (× 57). Fig. 3. Epoxy section of the smaller of the two early lesions examined by electron microscopy. Macrophages containing lipid vacuoles lie between the remaining myelin sheaths. Nuclei of the type seen at top (arrow) were found by electron microscopy to be packed with particles resembling paramyxovirus nucleocapsids (× 1200).

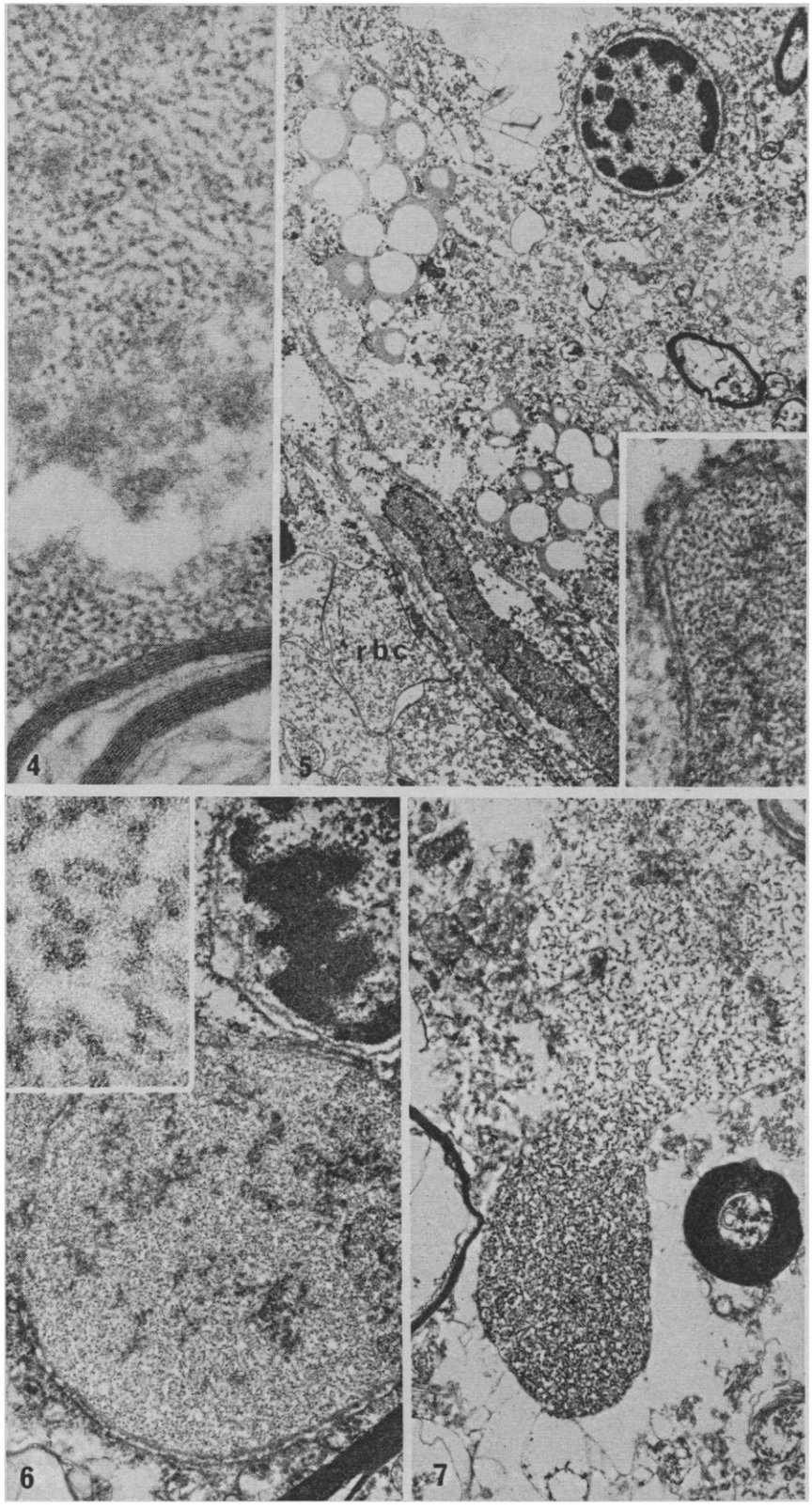

Fig. 4. A cytoplasmic aggregate of virus-like material adjacent to a myelin sheath (× 38,000). Fig. 5. Fine tubules filling an elongated nucleus in the Virchow-Robin space of the blood vessel at bottom left (*rbc*, red blood cell). Lipid vacuoles can be seen outside the vessel (× 3600). The inset shows detail of this nucleus (× 16,000). Fig. 6. A large nucleus filled with fine tubules and adjacent nucleus of normal appearance. This figure was obtained from the edge of the demyelinated zone in the smaller of the two active lesions (× 10,000). The inset shows detail of intranuclear tubules (× 120,000). Fig. 7. A large cytoplasmic fragment packed with nucleocapsid-like tubules in myelinated tissue at the edge of the larger of the two acute lesions (× 12,000).

chow-Robin space (Fig. 5), immediately outside the vessel, and among the nerve fibers (Fig. 6). In a few instances the cytoplasm of these cells contained lipid vacuoles.

In the larger of the two early lesions examined the same features were present, including cytoplasmic (Fig. 7) and nuclear tubules. In this lesion, however, numerous fine astroglial processes were seen in the demyelinated zone. In addition, prolonged scanning of both large subacute lesions and of chronic lesions revealed several isolated examples of cells containing similar tubular material.

The nuclear and cytoplasmic tubules in acute lesions described here closely resemble, in morphology and size range, the cytoplasmic parainfluenza nucleocapsids retrieved by cocultivation techniques from MS brain tissue by ter Meulen *et al.* (*3*). Other paramyxoviruses, including measles, have nucleocapsids 18 nm in diameter and with a similar morphology in tissue sections, and these observations are also consistent with the presence in early demyelinating lesions of nucleocapsids of measles or some other member of the paramyxovirus group (*6*).

Immunosuppressive therapy may have unmasked virus in acute lesions in this patient; previous ultrastructural studies of brain tissue from MS subjects, including the two brains from which an infectious parainfluenza virus was cultured, failed to detect virus material. However, virus material in this study was conspicuous only in two small early lesions, which would probably have been missed had routine sampling procedures been used. Therefore, virus material may prove to be a consistent feature of the early MS lesion.

The recovery by ter Meulen *et al.* (*3*) of a paramyxovirus from MS brain material and the present observations suggest the MS may be—like subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy, kuru, and Jakob-Creutzfeldt disease—a slow virus infection of the central nervous system. The histological changes in MS, however, resemble more closely the changes seen in experimental allergic encephalomyelitis (EAE), postinfectious encephalomyelitis, and rabies postvaccination encephalomyelitis than any of the known infectious leukoencephalitides (*7*); and others have suggested that if MS is related to a virus infection, a cellular immune response of the EAE type, provoked in some way by virus, might be involved. A feature of lesions in the present material was the

presence of intranuclear paramyxovirus-like particles in mononuclear cells infiltrating perivascular areas of active myelin breakdown. This has not been observed in other progressive virus diseases of the central nervous system, and it suggests that MS lesions may represent sites of endogenous infection due to periodic seeding of virus-bearing lymphocytes to the central nervous system. However, by analogy with EAE, some special property of the lymphocyte rather than the virus may determine the selective occurrence of lesions in central nervous system white matter. Immunologically specific transformation is required to activate certain latent virus infections of small lymphocytes (*8*); MS may involve a chance latent infection by paramyxoviruses of potentially encephalitogenic lymphocytes, which periodically transform and express virus on contact with central nervous system white matter (*9*).

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9. The patient's circulating lymphocytes, obtained 52 and 72 days before death, were cultured for up to 21 days with fetal calf serum, with and without phytohemagglutinin; electron microscopy of these cells did not reveal virus particles (R. G. Wright, personal communication). For this reason it is suggested that the infection is latent. Recent observations in canine distemper and Marek's disease suggest mechanisms that might be involved in subsequent demyelination in such a situation. H. Wiśniewski, C. S. Raine, W. J. Kay [*Lab. Invest.* **26**, 589 (1972)] described an EAE-like pattern of demyelination by hematogenous cells occurring in the presence of distemper virus in astrocytes. In Marek's disease, circulating lymphoid cells bearing Marek's disease herpes virus in a latent form [J. G. Campbell and G. N. Woode, *J. Med. Microbiol.* **3**, 463 (1970)] incite an EAE-like destruction of myelin in the absence of virus in neural tissue [J. W. Prineas and R. G. Wright, *Lab. Invest.* **26**, 548 (1972)].
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Motility of Macrogamonts of Eimeria magna (Coccidia) in Cell Culture

Abstract. *Amoeboid movement of spheroid or ovoid immature or mature macrogamonts within cultured Madin-Darby bovine cells usually began with the formation of a pseudopodium-like protrusion at the margin of the gamont. The protrusion enlarged as the gamont cytoplasm and nucleus moved into the protruded area. During movement, macrogamonts had an elongate shape. The body of the gamont was constricted as it moved through the cell cytoplasm or from one cell to an adjacent one. After movement had ceased, the gamont resumed its ovoid or spheroidal shape.*

Motility is characteristically present in various stages (sporozoites, merozoites, and microgametes) in the life cycle of *Eimeria* species and related sporozoa. The occurrence of motility has not been reported in other stages of the life cycle, except for the ookinete of malarial parasites (*1*). We recently observed motility in macrogamonts of the rabbit coccidium, *Eimeria magna*, in cell culture.

Monolayers of Madin-Darby bovine kidney cells (120th passage) were inoculated with merozoites obtained by scraping the mucosa of the lower two-thirds of the small intestine of rabbits inoculated 4½ and 5 days earlier with approximately 300,000 oocysts of *E. magna*. The mucosal scrapings were gently stirred with a glass rod, and the mixture was then rinsed in saline A (*2*) containing 5000 μg of dihydrostreptomycin and 5000 units of penicillin G per milliliter. The suspension was centrifuged at 200*g* for 1 minute, and the supernatant fluid, containing the merozoites, was removed. The merozoites were resuspended in minimal essential medium (MEM) in Earle's balanced salt solution without serum or antibiotics. They were counted with a hemacytometer, and 1 ml of MEM, containing approximately 300,000 or 400,000 merozoites, was inoculated into each Leighton tube, with a monolayer on a cover slip (10 by 35 mm). After a 6-hour incubation period at 37°C, 1 ml of MEM containing 5 percent fetal calf serum as well as 50 units of penicillin G per milliliter and 5 μg of dihydrostreptomycin per milliliter was added to each tube. At intervals of 12 hours for a period of 120 hours, cover slips were removed from Leighton tubes and examined in double cover slip preparations (*3*) with phase-contrast microscopy. To record macrogamont motility a filming speed of 120 frames per minute was used with 7242 Ektachrome EF 16-mm film for time-lapse cinemicrography and Tri-X Pan 35-mm film was used for still photomicrographs.

From 36 to 120 hours after inoculation, some intracellular merozoites had developed to micro- and macrogamonts by a process similar to that described previously for *E. magna* (*4*). Simultaneously, other merozoites developed into schizonts with 2 to approximately 500 merozoites. At 36 hours, young macrogamonts were distinguishable from microgamonts by their relatively large size, by the large nucleus and nucleolus, and by the presence of plastic granules in their cytoplasm. At 72 hours, intermediate macrogamonts were ovoid in shape, and 20 live specimens averaged 17 by 12 μm; the nucleus and nucleolus were 6 and 2 μm in diameter, respectively. Mature macrogametes, which were seen at 72 to 120 hours, were similar to the intermediate stages, except that they were larger (27 by 21 μm).

Intracellular movement of young and intermediate macrogamonts was seen at 72 and 84 hours, whereas motile mature macrogamonts were seen at 84 and 96 hours. Usually, the motile macrogamonts moved within the cytoplasm of the host cell and, occasionally, moved into an adjacent cell. During movement, gamonts became elongate. A small pseudopodium-like protrusion developed at the margin of the gamont;