Experimental Parainfluenza-Type-1-Virus-Induced Encephalopathy in the Adult Mouse

An Ultrastructural Study of Early Lesions

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The adult mouse inoculated intracerebrally with 6/94 strain of parainfluenza type 1 virus developed selective degenerative lesions in cerebral white matter. Ultrastructurally, the infiltration of mononuclear cells, mostly lymphoid cells, apparently preceded the alterations of white matter parenchyma. The prominent feature of the white matter lesion was a lytic degeneration of both axon and myelin that seemed to be triggered by the mononuclear cell infiltration. Nucleocapsids of paramyxovirus were found only in ependymal cells and at the very early stages of the infection. It is suggested that the mechanism of the white matter degeneration might be that of a virus-induced cell-mediated immune response directed at both the axon and myelin. (Am J Pathol 79:335–346, 1975)

PARAINFLUENZA TYPE 1 VIRUSES, both Sendai[1] and 6/94[2] strains, have been shown to induce chronic degenerative lesions in the white matter of adult mouse brain after intracerebral inoculation.[1,3,4] In both strains, the viral antigens were detected only in ependymal cells and only at the very early stages of the infection, and the degenerative lesions seemed to progress in the absence of viral replication or viral antigens.[3] In this sense, the parainfluenza-type-1-virus-induced white matter lesion is unique among virus-induced encephalopathies.

The present study was undertaken to further elucidate the pathogenic mechanism of parainfluenza-type-1-virus-induced white matter lesions. This paper presents the results of an ultrastructural study on the early changes in white matter lesions of adult mouse brain after intracerebral inoculation of 6/94 virus.

Materials and Methods

Animals

Male and female Swiss albino mice, 6- to 8-weeks-old, of the ICR strain were purchased from Flow Laboratories, Rockville, Md.

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Virus

One stock of 6/94 virus was used. The virus was originally isolated from cultured brain cells obtained from 2 patients with multiple sclerosis [2] and had been continuously passaged in embryonated hen's eggs. Infectivity (in egg-infectious dose) of the stock virus was $10^{7.0}$ EID_{50}/ml, and the hemagglutination titer was 1:1024.

Inoculation

A dose of 0.03 ml of 1:10 dilution of the stock virus was inoculated into the right cerebral hemisphere.

Tissue Processing and Examination

For the electron microscopic study, animals were sacrificed 2, 3, 4, 5 and 7 days after inoculation. Various portions of the brain (paraventricular tissue, corpus callosum, cerebral cortex and Ammon's horn) were fixed in a 3.5% glutaraldehyde solution adjusted to pH 7.3 with 0.1 M phosphate buffer. They were postfixed in 1% osmium tetroxide solution adjusted to pH 7.3 with Millonig's buffer,[5] dehydrated and embedded in epoxy resin. Thick sections were stained with toluidine blue and examined on a light microscope. Thin sections were stained with uranyl acetate and lead citrate and examined on a Hitachi HU-11 electron microscope.

Tissues not used for electron microscopy were used for parallel virologic and routine histologic studies.[3,4]

Results

Light Microscopy

Although animals inoculated with the 6/94 virus showed no neurologic signs clinically, encephalitis developed in all of the inoculated animals. The severity of the lesions varied from animal to animal.

Acute inflammatory changes developed in cerebral white matter and in meninges and subependymal regions. In the white matter, mononuclear cells were seen as a diffuse infiltration into the parenchymal tissue and as perivascular cuffing (Figure 1A). The acute inflammation was most pronounced on the fourth day, when a faint staining or disintegration of myelin and focal rarefaction of white matter was seen around the severe inflammatory changes (Figure 1B). The intensity of the myelin involvement seemed to parallel the severity of mononuclear cell infiltration. At this stage, focal detachment of ependymal cells was also conspicuous. On the fifth to seventh days, inflammatory cell infiltration became less extensive, but the involvement of parenchymal tissue of the white matter was progressing and focal necrosis became manifest.

Inflammatory changes were not found in the cerebral cortex except for an infrequent appearance of perivascular cuffing around the larger veins.

Electron Microscopy

Viral Infection

Nucleocapsids of paramyxovirus were observed exclusively in ependymal cells and were detected only in specimens taken on the second and third days after inoculation. The nucleocapsids, distributed in the cytoplasm of ependymal cells, were coated with thin amorphous materials (Figures 2 and 3). No virus was seen budding from the cells bearing the nucleocapsids.

Changes in the Ependymal Region

Morphologic alterations of the ependymal cells were observed as early as the third day. Cytoplasmic vacuoles and separation from neighboring cells were frequently seen (Figure 4). Some of the infected ependymal cells underwent necrosis. Partial excoriation of the ependymal and subependymal layers was seen after the fourth day, and it was exaggerated by the inflammatory cells infiltrating the subependymal region. The underlying white matter showed marked rarefaction, but myelinated nerve fibers remained largely unchanged. Surviving ependymal cells contained increased glycogen granules on the fourth to seventh days (Figure 4) when astrocytes having numerous glycogen granules and fibrils proliferated in the subependymal region.

Mononuclear Cell Infiltration

The earliest change in the white matter was the infiltration of mononuclear cells. The identification of the cell type of the mononuclear cells was difficult even by electron microscopy. The majority of the mononuclear cells appeared to be lymphoid cells, characterized by various amounts of cytoplasm with a less electron-dense ground matrix, numerous polyribosomes, scanty endoplasmic reticulum and pseudopodia-like cytoplasmic projections (Figures 5–8). They were indistinguishable from small stimulated lymphocytes,[6] hemocytoblasts [7] or immunoblasts.[8] Typical lymphocytes and monocytes were infrequently found, and granular leukocytes or plasma cells were extremely rare. Plasma cells were encountered in the perivascular region after the fourth day. Macrophages, distinguishable from the above-mentioned lymphoid cells, were frequently seen in destructive lesions of white matter, and they contained phagocytic debris.

Changes in the White Matter Parenchyma

The first response of parenchyma of the white matter to the inflammation was a slight swelling of astrocytic processes with increased glycogen

granules (Figure 5). This swelling was apparently a nonspecific edema since these changes were also seen in areas distant from the mononuclear cell infiltration. Alterations of myelinated axons were frequently encountered in the immediate vicinity of the mononuclear cells (Figures 6 and 8). The most conspicuous findings in myelinated axons were degenerative or lytic alterations which seemed to be initiated simultaneously in both axon and myelin. Axonal changes (characterized by swollen axons with an accumulation of mitochondria, membranous dense bodies, vesicular elements, neurofilaments or neurotubules) were noted around the mononuclear cells (Figures 6 and 8). These axonal elements underwent granular disintegration (Figures 9 and 10). Mononuclear cells engulfed degenerating axons (Figure 9).

The altered axons were usually accompanied by various degrees of myelin degeneration (Figures 7, 9 and 10). Two different processes of myelin destruction were seen: a) extracellular lysis of myelin in which the myelin sheaths were partially dissolved into amorphous substances with vesicular debris (Figures 7 and 10) and there was an extracellular accumulation of electron-dense masses with cell debris around the denuded axons (Figures 6 and 11) (these electron-dense masses were probably myelin debris mixed with plasma precipitates); b) phagocytosis of myelin by mononuclear cells (Figures 7, 10 and 12). In the more advanced lesions, most of the cells participating in phagocytosis of myelin were mononuclear macrophages which were easily distinguishable from lymphoid cells (Figure 12). Despite careful search, processes similar to the typical "peeling or stripping" of myelin lamellae by mononuclear cells [9] were never seen.

The oligodendrocytes remained unchanged, and no special interaction between oligodendrocytes and mononuclear cells was observed.

Discussion

The present study revealed that: a) viral structures were confined to ependymal cells and were seen only in the very early stages of infection; b) infiltration of mononuclear cells, mainly consisting of lymphoid cells, preceded the alteration of parenchymal tissue of cerebral white matter; c) the prominent feature of the white matter lesion was that of a lytic degeneration of both axon and myelin which seemed to be triggered by mononuclear cell infiltration.

The absence of viral structures in cells other than the ependymal cells corresponds to the results of a parallel immunofluorescent study.[3] Myxoviruses or paramyxoviruses usually do not spread beyond ependymal cell layer in adult rodents after intracerebral inoculation. In in-

fluenza virus and mumps virus infections,[10] virus antigens can be detected only in ependymal cells, but no encephalitis develops in brains of adult rodents. Unlike these viruses, SSPE virus can spread into brain parenchyma of adult rodents and replicate in neurons and glias, and encephalitis occurs predominantly in grey matter.[11] In the present study, degenerative lesions in white matter developed without evidence for virus replication or persistence of virus antigen and progressed over the extended period of time after the subsidence of inflammatory changes.[3,4] Similar degenerative white matter lesions without virus replication have been reported by Mims and Murphy [1] in adult mouse brain after inoculation of Sendai strain of parainfluenza type 1 virus. Thus, parainfluenza type 1 virus infection in adult mouse brain has a unique feature of virus–brain-cell interaction among myxovirus- and paramyxovirus infections.

At present, the mechanisms involved in the degeneration of white matter reported in this study are unknown. Although not excluded, the possibility that some viral components can inflict damage to axon and myelin is unlikely, since the degenerative lesion was initiated in the immediate vicinity of mononuclear cell infiltration and not in the subependymal region where the viral components could affect white matter most intensely. For this reason (and the evidence that ultraviolet-inactivated virus can induce similar white matter lesions in absence of ependymitis [4]) the lesion is apparently not a simple consequence of ependymal cell necrosis.

The cell-mediated immune reaction has been suggested as the mechanism of demyelination in such diseases as experimental allergic encephalomyelitis [9] or neuritis,[12] canine-distemper-virus-induced encephalomyelitis, [13] and herpes-virus-induced neuritis (Marek's disease) [14] in which the outstanding feature is primary demyelination initiated by mononuclear cell infiltration. The morphologic events occurring in the 6/94-virus-induced white matter lesion were very similar but not identical to those seen in these demyelinating diseases. The prominent feature of parenchymal changes was not primary demyelination but degeneration of both axon and myelin. This difference could result if the immune-mediated reaction was directed at myelin or myelin-supporting cells in the demyelinating diseases, and at both axon and myelin in the 6/94-virus-induced encephalopathy. Otherwise, this difference is simply a matter of the severity of inflammation, since both axon and myelin are always involved in the demyelinating diseases, especially in cases where the lesions consist of massive or severe mononuclear cell infiltration.[9,13,14] The important thing is that, common to both, the parenchymal changes were initiated by mononuclear cell infiltration. In this respect, 6/94-

virus-induced encephalopathy can be regarded as a model for some human leukoencephalopathies in which mononuclear cell infiltration is regarded as the earliest change [15] and a viral etiology is suspected but no virus antigen can be demonstrable. The possible involvement of the immune-mediated reaction in the 6/94-virus-induced encephalopathy is currently under investigation.

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Acknowledgments

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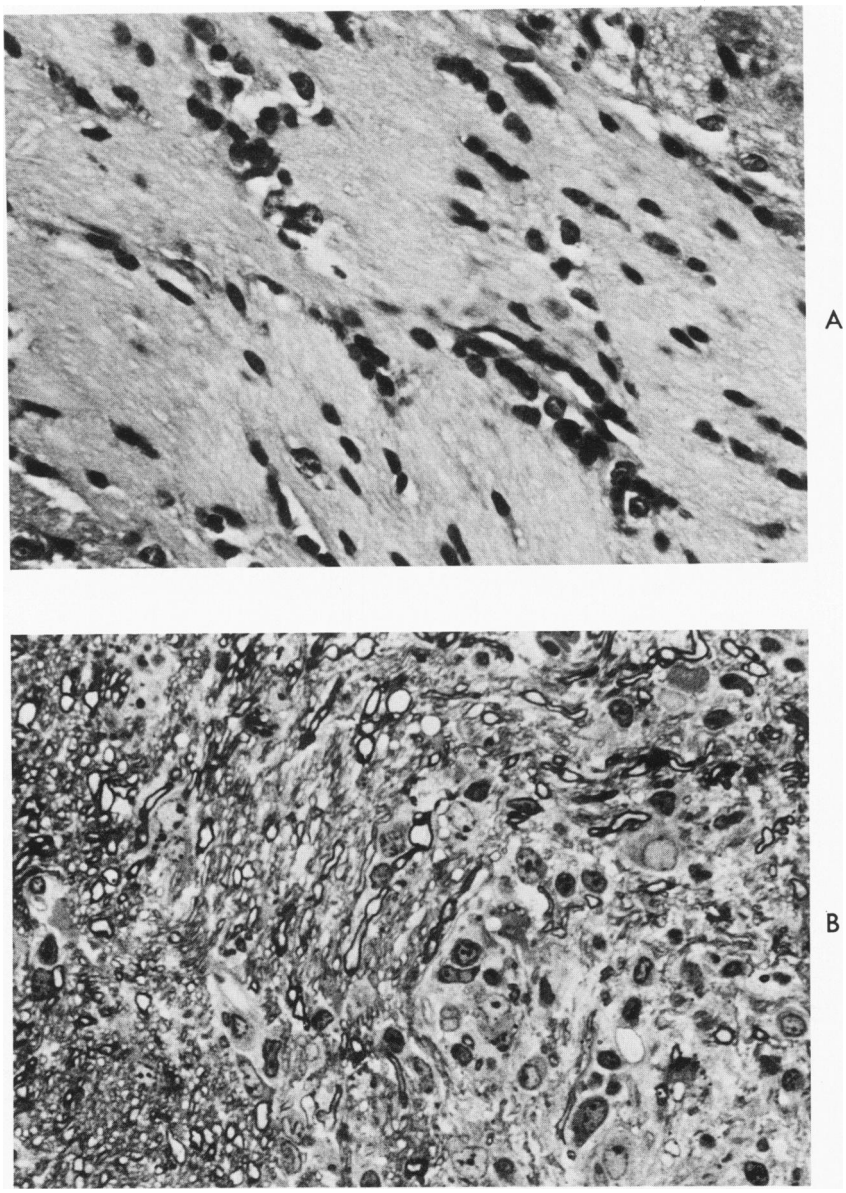

Fig 1—Light micrographs of the lesion in corpus callosum. **A**—Mild mononuclear cell infiltration, second day (Paraffin embeddment, hematoxylin and eosin, × 400). **B**—Heavy mononuclear cell infiltration, and faint staining and disintegration of myelin (lower right), fourth day (Epon embeddment, toluidine blue, × 430).

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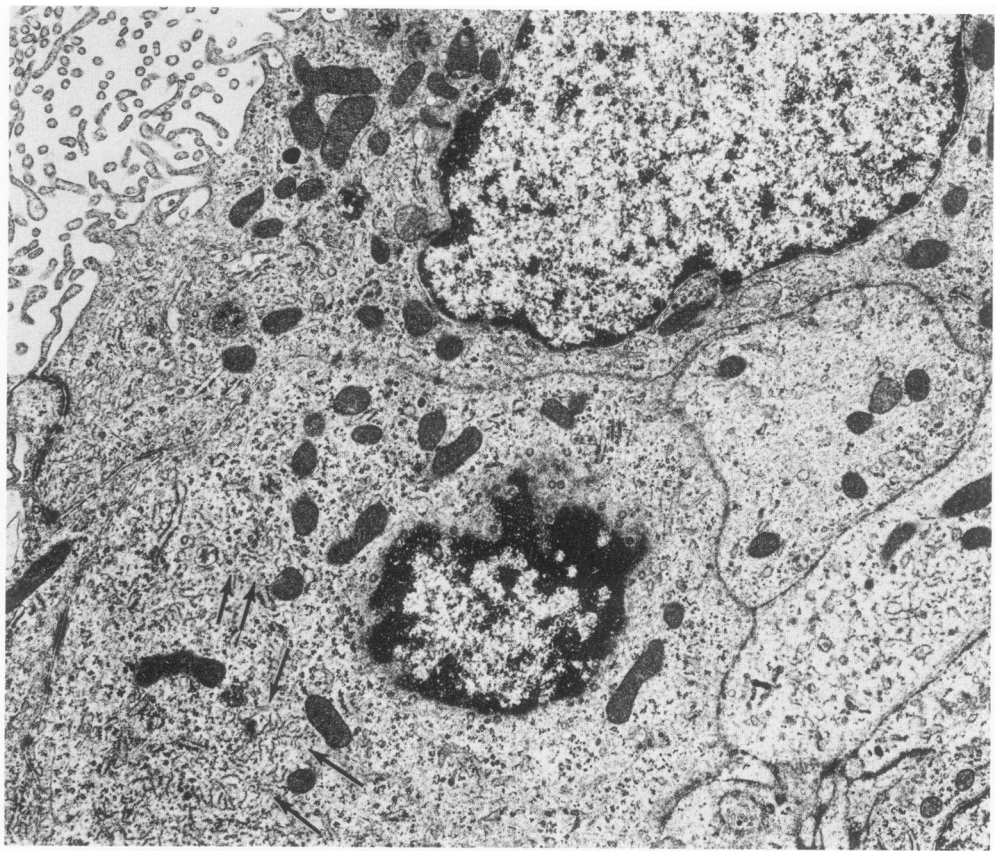

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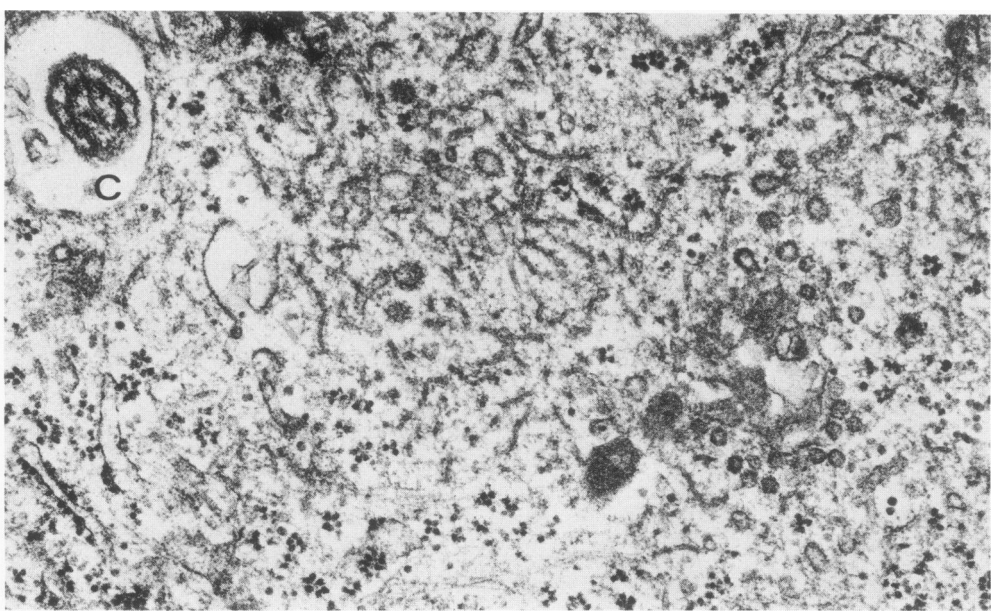
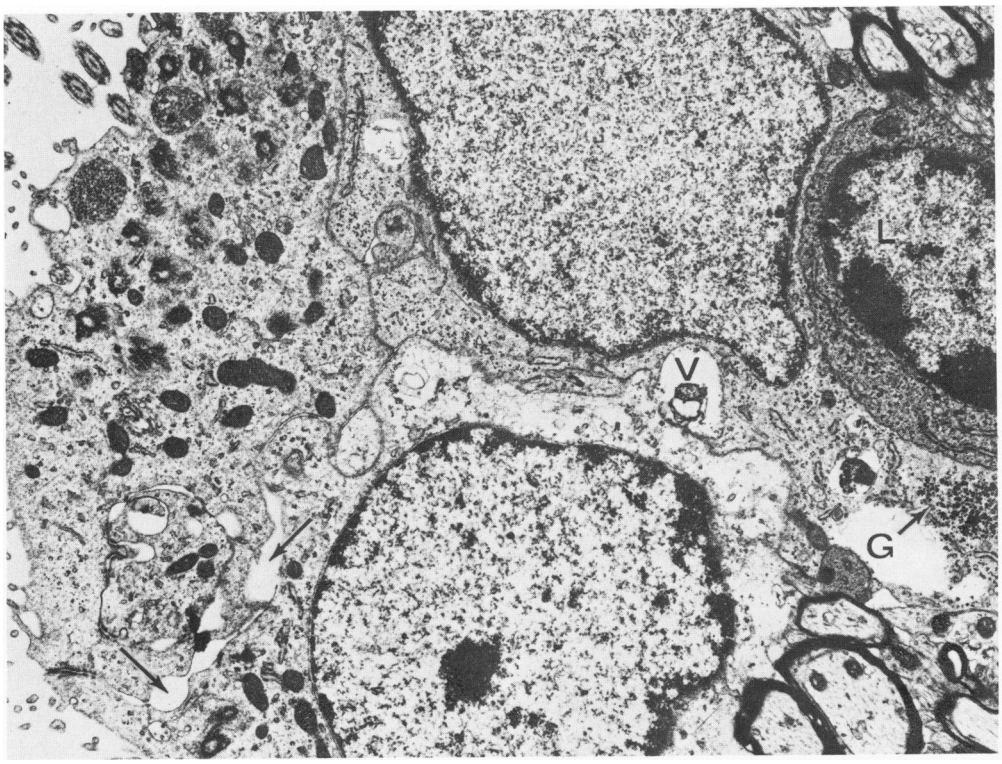


Fig 2—Electron micrograph of ependymal cells. Nucleocapsids are seen in the cytoplasm of ependymal cells *(arrows)*, third day (× 11,200). **Fig 3**—Higher magnification of nucleocapsids in the ependymal cell, third day. *C* = cilia (× 48,000).



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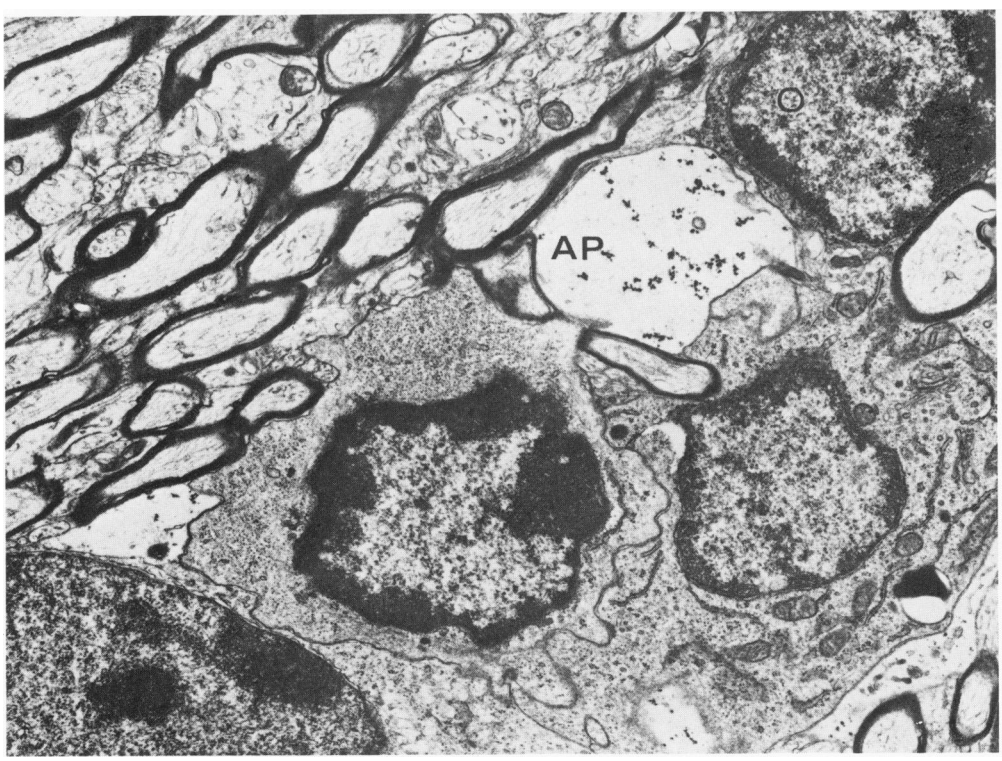

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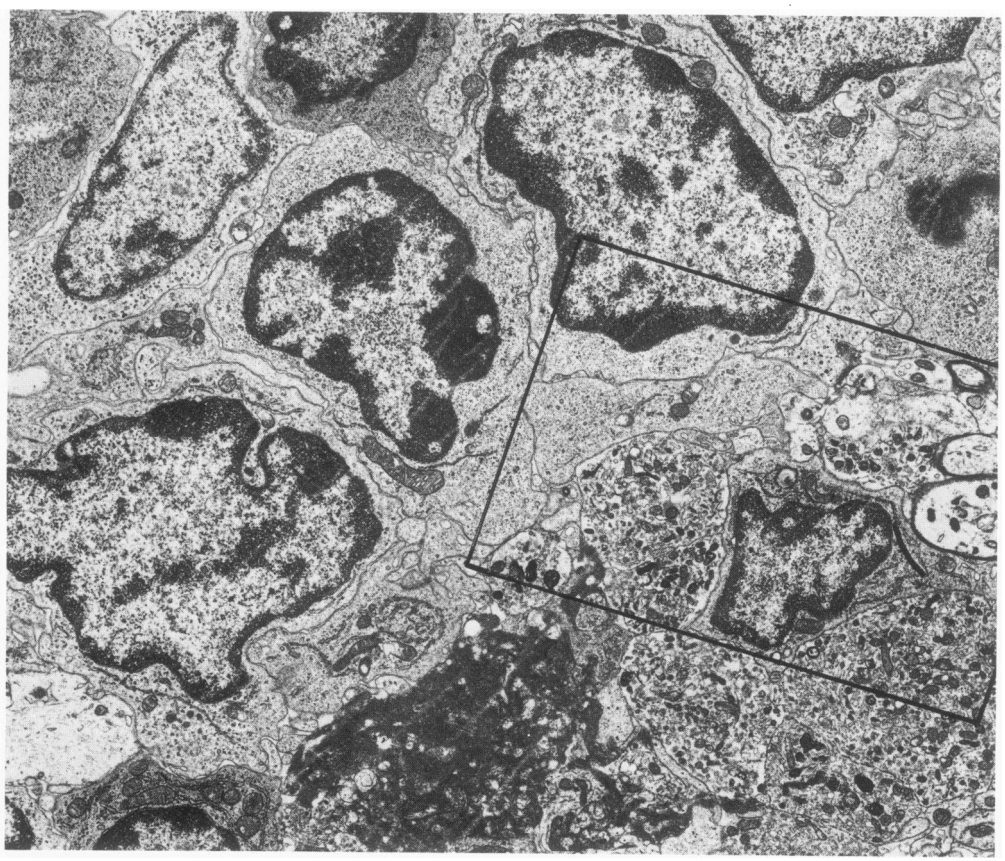
Fig 4—Vacuole formation (*V*) and partial detachment (*arrows*) of ependymal cells, fourth day. *G* = glycogen, *L* = lymphocyte (× 10,200). **Fig 5**—Lymphoid cell infiltration in white matter. Astrocytic processes (*AP*) with increased glycogen is slightly swollen, second day. *O* = oligodendrocyte (× 10,500).



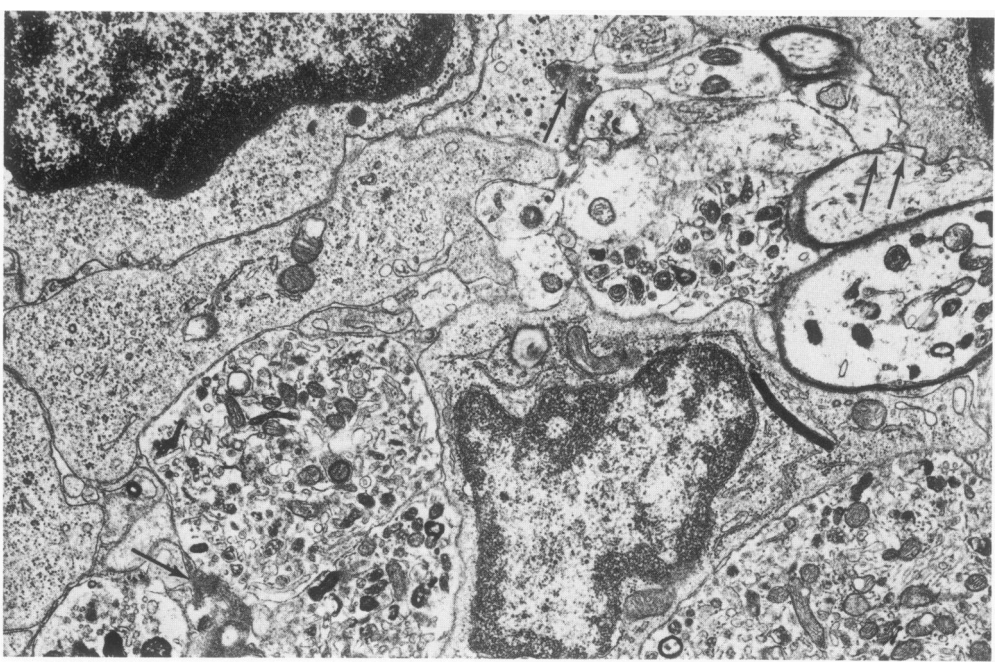
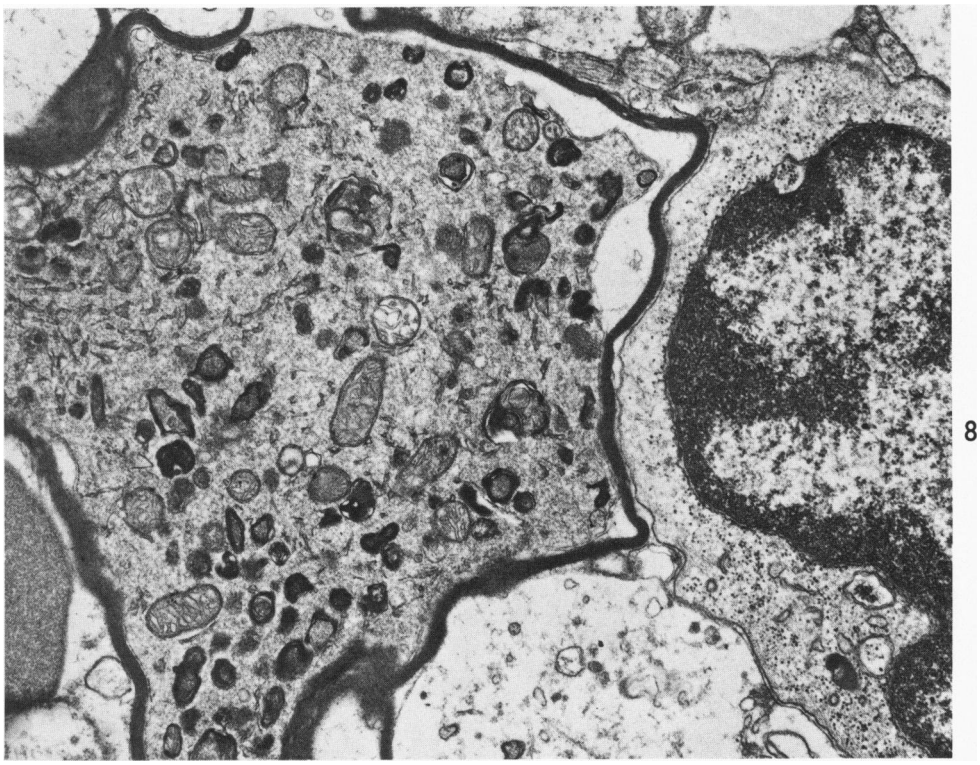


Fig 6—Heavy mononuclear cell infiltration in white matter. Many axons undergoing degeneration are closely surrounded by lymphoid cells. Electron-dense masses with cell debris are seen in the extracellular space around the degenerated axons, third day (× 8800).
Fig 7—Higher magnification of the outlined area in Figure 6. Some axons are partially demyelinated (*double arrows*). Lymphoid cell contains myelin debris. *Single arrows* indicate electron-dense masses in the extracellular space (× 18,700).



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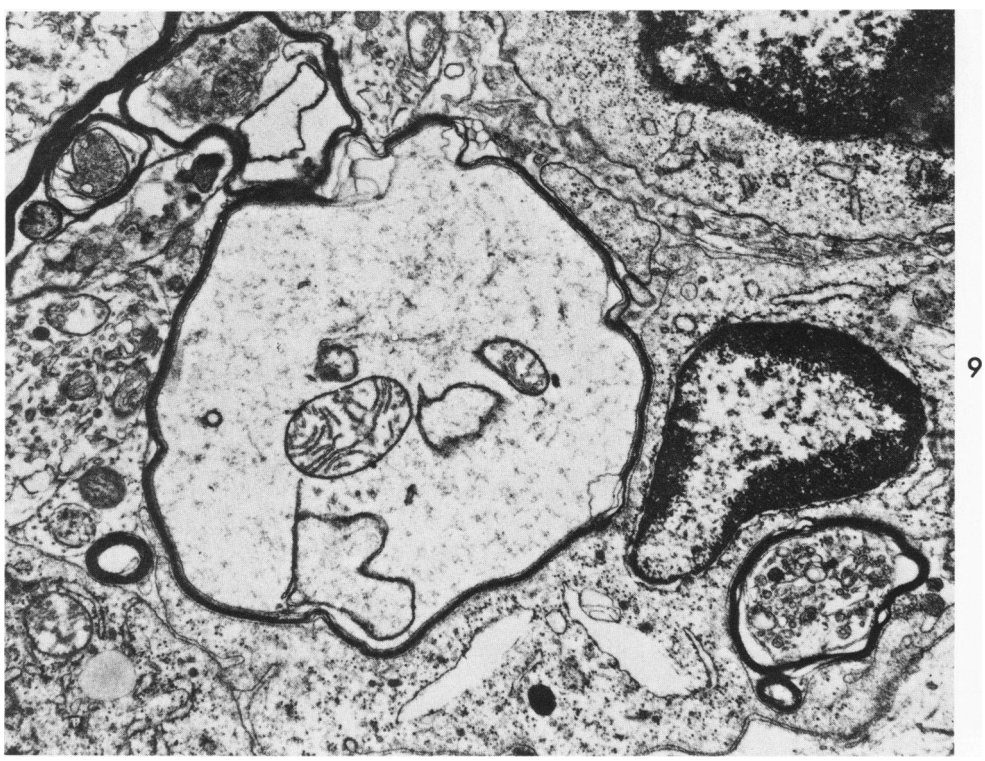

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Fig 8—Axonal degeneration around lymphoid cell, third day (× 17,800). **Fig 9**—Many axons are undergoing granular disintegration. A mononuclear cell contains a degenerating myelinated axon, third day (× 16,000).

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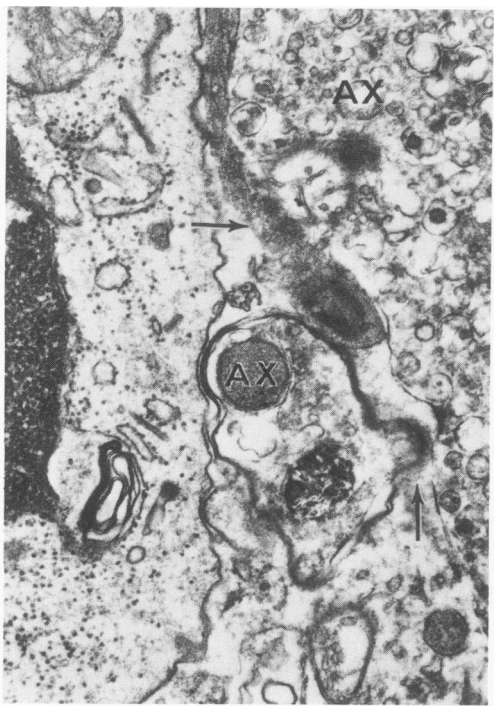

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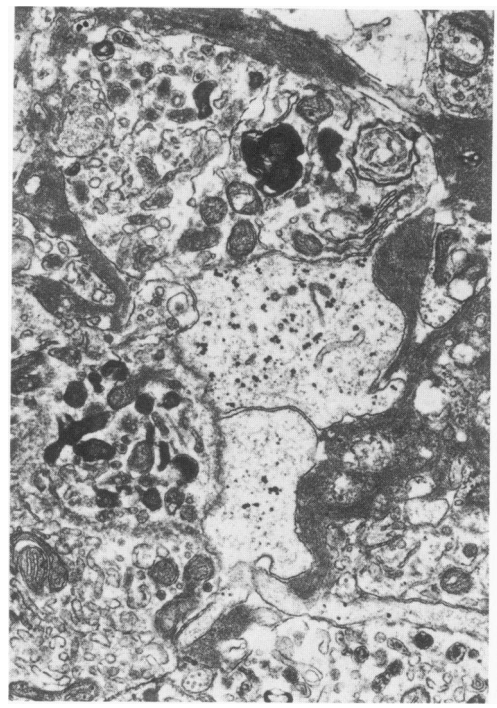

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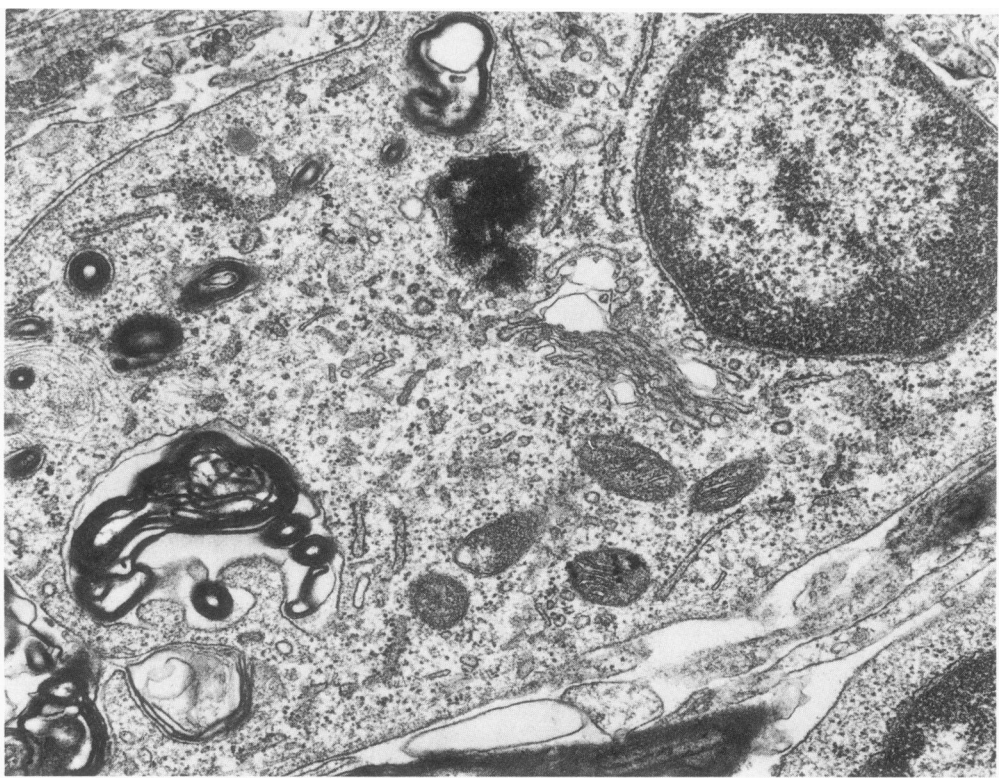

Fig 10—Myelin sheaths of axons *(AX)* undergoing granular disintegration is dissolved into electron-dense masses (*arrows*). Lymphoid cell contains myelin debris, third day (× 30,600). **Fig 11**—Extracellular electron-dense masses around denuded degenerating axons, third day (× 19,800). **Fig 12**—Mononuclear macrophage phagocytosing many myelin debris, fifth day (× 16,000).