Uncommon Morphologic Features in Subacute Sclerosing Panencephalitis (SSPE)

Report of Two Cases with Virus Recovery from One Autopsy Brain Specimen

Joseph C. Parker, Jr, MD, Gordon K. Klintworth, MD, PhD, Doyle G. Graham, MD and John F. Griffith, MD

In 1933 Dawson[1] drew attention, in this journal, to an encephalitis that was typified by intranuclear inclusion bodies. Subsequently, the disease became widely recognized as an entity, and by 1969 more than 100 cases had been reported from the United States alone.[2] The designation subacute sclerosing panencephalitis (SSPE) is the currently accepted term for this condition, although historically it has been known as Dawson's encephalitis, Van Bogaert's disease, subacute sclerosing leukoencephalitis, nodular panencephalitis, subacute inclusion body encephalitis, encephalitis of Pette-Döring, and diffuse encephalitis with sclerosing inflammation of the hemisphere white matter.[3]

This rare disease of childhood usually presents with the insidious onset of intellectual deterioration, inappropriate behavior and periodic myoclonic movements of the extremities and trunk. Typically, the clinical course is one of progressive impairment in motor and mental skills, leading to death in several months. Occasionally, patients have survived for 5–9 years and there are even rare reports of recovery.[4] The cerebrospinal fluid is usually acellular with normal glucose and total protein levels, but a modest lymphocytic pleocytosis and a moderate rise in protein may be observed. It usually contains an elevated amount of gamma globulin, which may be measured directly or reflected in a paretic (first zone) colloidal gold curve.

Although Dawson suspected a viral etiology for this disease, the measles virus was not implicated until 1965, when several laboratories reported finding filamentous tubular structures, with the electron microscope, in brain biopsy material.[5–13] These particles appeared to be iden-

From the Departments of Pathology, Pediatrics and Medicine (Neurology), Duke University Medical Center, Durham, North Carolina.

Supported in part by Grant 2T1-NB-5212 from the US Public Health Service and by Grant 383-5587 from the National Multiple Sclerosis Society.

Accepted for publication Aug 3, 1970.

Address for reprint requests: Dr. D. G. Graham, Department of Pathology, Duke University Medical Center, Durham, North Carolina 27706.

tical to those seen in tissue cultures inoculated with measles virus.[14] This association was reinforced by Connolly *et al*,[16] who found markedly elevated titers of measles antibodies in the serum and cerebrospinal fluid, and by the rising measles antibody titers observed during the course of the disease.[6,15–18] Additional evidence was provided by the fluorescent antibody technic, which localized measles antigen in the neurons and neuroglia of brain biopsy specimens.[7,16,19] Finally, in 1969 the measles virus was recovered from several cases of SSPE by independent investigators.[20,23]

Since the condition first became recognized, additional clinical and morphologic observations have broadened our concept of the disease. This report describes 2 patients, both of whom serve to emphasize certain of the uncommon clinical and morphologic features of SSPE. In one patient, the virus was recovered from the brain at postmortem examination.

Case Studies

Case 1

An 11-year-old, previously healthy girl (DU No. H49249) developed gradual intellectual deterioration, an unsteady gait, and dysarthria, over a period of several months. The cerebrospinal fluid (CSF) was acellular with a total protein content of 42 mg/100 ml, a markedly increased level of gamma globulin (18 mg/100 ml) and an elevated measles virus neutralizing antibody titer (1:256). Paroxysmal epileptic discharges, emanating from the left frontal region, were evident on the electroencephalogram (EEG). Psychologic evaluation indicated a full-scale IQ of 56.

The patient was admitted to the hospital 1 month later with "dizziness", occasional diplopia, significant cerebellar dysfunction and profound intellectual disability. When her condition deteriorated further, she was treated with therapeutic doses of intramuscular ACTH. Over the ensuing weeks she became less responsive, unable to talk and died approximately 7 months after the clinical onset of her disease.

She had no known history of measles but had received the live attenuated measles vaccine (Edmonston B strain, Eli Lilly and Co) with 0.5 ml gamma globulin without incident, approximately 3 years before the onset of her symptoms.

Case 2

A 12-year-old boy (DU No. H76774) suddenly developed progressive visual impairment. Two weeks later, physical examination was unremarkable apart from bilateral visual loss and focal retinal inflammatory lesions. Subsequently, he developed rapidly evolving lethargy and stupor and finally became comatose and unresponsive. For the 2 months prior to death, the patient was treated with varying doses of corticosteroids. The initial cerebrospinal fluid was acellular with a total protein content of 69 mg/100 ml. The gamma globulin was 52 mg/100 ml. The measles virus neutralizing antibody titers, both in the CSF and serum, were markedly elevated—1:256 and 1:4096, respectively. The EEG showed diffuse bilateral slowing, and the pneumoencephalogram indicated mild dilatation of both lateral ventricles. The patient developed increased intracranial pressure, which was controlled by repeated intravenous administrations of mannitol. He became hyperther-

mic and hypertensive and developed intermittent decerebrate posturing, electrolyte imbalance and upper gastrointestinal bleeding. He died suddenly, approximately 3 months after the onset of symptoms.

The patient had no known history of measles, although his two sisters had documented rubeola when he was 5 years old. Five months before the clinical onset of his fatal illness, he received the attenuated measles virus vaccine, Edmonston B strain (Phillips Roxane Pharmaceutical Co), with 0.5 ml of gamma globulin. There was no apparent reaction to this vaccine.

Postmortem Studies

In both children, autopsy examination included the brain, spinal cord and eyes. A focal, acute bronchopneumonia was seen in each patient. The remainder of the significant abnormalities were confined to the central nervous system.

Case 1 had a diffusely, symmetrically swollen brain that weighed 1450 g. Slightly widened gyri and narrowed sulci were accompanied by mildly compressed ventricles. The gray-white junction was generally obscured. In Case 2, the brain weighed 1500 g and, in addition to cerebral edema, numerous areas of the cerebral cortex exhibited laminar necrosis, which was especially prominent in the temporal lobes (Fig 1 and 2). A few small perivascular hemorrhages were scattered throughout the cerebral hemispheres. Foci of subcortical demyelination were particularly prominent in the right occipital lobe (Fig 3).

Light Microscopy.

Representative portions of all organs, including multiple sections of the central nervous system, were processed for light microscopy. Both patients had multiple lesions widely dispersed throughout the gray and white matter of the nervous system, without predisposition for any particular region. Aside from nonspecific features of viral encephalitis, such as perivascular and leptomeningeal lymphocytic infiltrates, there were areas of gliosis associated with a patchy demyelinization (Fig 3). One of the unusual features of both of these cases was the intense involvement of the cerebral cortex. In one patient particularly (Case 2), this was evident with the naked eye as laminar necrosis (Fig 1 and 2) while in the other, this was seen only microscopically. These areas were widely scattered throughout the cerebral cortex and were not restricted to those regions most susceptible to hypoxia. The cerebellar cortex and anterior horns of the spinal cord were involved in Case 1, while in the other, retinal lesions were seen. The cerebellar lesions included loss of Purkinje cells, an increase in Bergmann's astrocytes, and occasional glial nodules (Fig 4). The most striking morphologic feature within the neurons and neuroglia, at all these levels, were several distinct types of

intranuclear and intracytoplasmic eosinophilic inclusions. The inclusions were single or multiple and varied in shape and size. Some rounded intranuclear inclusions (Cowdry Type B) resembled the nucleolus, but were eosinophilic rather than basophilic; others possessed a clear halo between the inclusion and the marginal nuclear chromatin (Cowdry Type A), while other inclusions filled the entire nucleus and abutted against a thin rim of peripheral chromatin (Fig 5–8). There were transitional forms between the various types of intranuclear inclusion bodies.

Electron Microscopy

Portions from multiple sites of the central nervous system were collected for electron microscopy from both cases shortly after death (1–6 hours). These tissues were immediately sectioned into cubes less than 1 mm on a side, fixed in cold 1% Veronal-buffered osmium tetroxide (pH 7.4) for 1 hour and cold sodium cacodylate-buffered glutaraldehyde (pH 7.4) for 10–12 hours, and then postfixed in 1% Veronal-buffered osmium tetroxide for 1 hour. All tissues were dehydrated in graded alcohols, infiltered with propylene oxide and embedded in Epon. Thin sections were cut with a Sorvall Porter-Blum microtome, mounted on 200-mesh copper grids and stained for 6 minutes with 4% magnesium uranyl acetate in distilled water, rinsed with distilled water (2 changes) and then stained for 4 minutes with lead citrate (pH 12) at room temperature.[24] Electron microscopic observations were made with a RCA EMU-3G microscope at 50 kV. Companion sections, cut at 2 mμ, were stained with thionine-azure blue for orientation.[25]

In both cases, neurons and neuroglia contained dense intranuclear aggregates of randomly disposed interwoven tubular structures 150–269 Å in width (Fig 14). These sometimes replaced most of the nuclear contents and were surrounded by peripherally marginated chromatin (Fig 9). In less severely involved cells, they were evident only within portions of the nucleus. Rarely, collections of these structures were identified within the cytoplasm (Fig 10). Some nuclei contained oval or rounded bodies (1–3 μ in diameter) characterized by concentrically laminated membranes, or electron-dense masses of closely aggregated material (nucleoliform bodies). These bodies were particularly numerous in Case 1.

Viral Studies

In Case 2, representative specimens from the right frontal and left

parietal regions, cerebellum and spinal cord were obtained aseptically less than an hour after death and placed in sterile medium (Hank's balanced salt solution containing penicillin (100 units/ml), streptomycin (100 μg/ml) and amphotericin (50 μg/ml). After mincing with scissors, trypsin (0.25%) was added and allowed to act until the specimens were reduced to single cells and small particles. An approximate inoculum of 1 million cells/cu mm was placed into a number of glass tubes and flat-bottomed plastic flasks and maintained in an incubator at 37 C. The cultures were maintained at constant pH (approximately 6), supplemented with fresh nutrient media and, when a confluent monolayer developed, were subcultured. Cultures from repeated passages were maintained in the same manner. At intervals, cultured cells were dispersed mechanically, and centrifuged at 800 rpm for 15 minutes. The resulting pellet was fixed in sodium cacodylate-buffered glutaraldehyde (pH 7.4) at 5 C for about 16 hours, postfixed in Veronal-buffered osmium tetroxide for 1 hour, and then processed as described for electron microscopy.

With the methods usually employed to isolate viruses, no organisms were recovered. However, cultured brain cells from Case 2 (mainly fibroblast-like cells) remained unchanged in morphology until approximately 3 months after primary culture when small syncytia first appeared. These increased in size and number in subsequent cultures (Fig 11 and 12). Electron microscopy of these cultures, at this time, revealed numerous nuclear and cytoplasmic filamentous tubules identical to those seen in the postmortem brain material (Fig 12, 13 and 15).

These cell cultures were mechanically disrupted and overlaid onto AH-1 (GMK) cell cultures (a continuous line of grivet monkey kidney). Typical measles virus cytopathic effect (CPE) appeared within 5 days, and gradually progressed to involve most of the cell sheet. These and the primary brain culture were subsequently stained by the indirect immunofluorescent technic, using acute and convalescent measles antisera and then fluorescein-labeled rabbit antihuman gamma globulin. Brilliant yellow-green nuclear and cytoplasmic fluorescence localizing the measles antigen was seen in syncytial giant cells; control, uninfected cultures and infected material treated with acute-phase sera showed only nonspecific fluorescence.

Attempts to recover cell-free virus from these cultures by co-cultivation and the overlay technic were unsuccessful. Cultured brain cells with advanced CPE were then disrupted and inoculated intracerebrally into suckling hamsters. On recultivation of the inoculated hamster brain, cell-free virus was recovered that produced typical measles CPE in AH-1

(GMK) and human placental cell cultures. This virus has now been serially passed in these cells and has been identified as the measles virus by neutralization with known measles antibody.

Discussion

Some of the typical morphologic features of SSPE, which these cases demonstrate, include neuron loss, perivascular round cell infiltration, gliosis, and inclusion bodies. The inclusion bodies are intranuclear and intracytoplasmic and appear within neurons and neuroglia. Their ultrastructural correlate is recognized as filamentous microtubules identical to those seen in both of these patients.

The presence of lesions in the retina, spinal cord and cerebellum is unusual for this disease.[26–30] Though retinal lesions are probably much more common than the literature suggests, they are not often recognized clinically and seldom demonstrated pathologically. The lesions are single or multiple, and usually involve the macula or perimacular regions. They consist of patchy areas of inflammatory edema which, as occurred in this case, may appear early before the central nervous system disease is obvious. In this instance the retinal involvement was clinically apparent for only a few weeks and would not have been appreciated if ocular symptoms had not heralded his disease. In view of this observation and the increasing number of reports in the literature [26,27] one might predict that retinal disease is the basis for some of the visual symptomatology that, in the past, had been attributed to the cerebral process.

The cerebellar and spinal cord involvement are not explicable simply on the basis of the tempo of the inflammatory process. Other cases with clinical courses lasting a few months to many years have not demonstrated lesions at these levels. This underscores our naïvité about the pathogenesis of this disease even though certain observations have been made correlating morphologic changes with time. After a prolonged course, gliosis and demyelination are usually pronounced, while the inclusion bodies are generally more readily identified in individuals with a short clinical illness.[31] In fact, there are reports of inclusion bodies being found in cerebral biopsies prior to death, but not in postmortem examinations.[32,33] Demyelination does not always appear to be a sequel to cortical involvement, especially in those cases of short duration, but may reflect involvement of the oligodendroglia, which commonly contain intranuclear inclusions.[34,35] Myelin loss is commonly seen in focal inflammatory lesions in the white matter, but this is a necrotizing process resulting in loss of all cellular elements, including myelinated axons.

Though the gray matter is commonly involved in SSPE [1,28,36-39] laminar necrosis of the degree noted in Case 2 has seldom been reported.[39] As laminar necrosis results from cerebral hypoxia, its occurrence in SSPE suggests alternate explanations for this lesion. Since the metabolic demands of an inflamed brain are increased and it is more vulnerable to injury, it may be that the cerebral lesion resulted from an ischemic-hypoxic insult that could not be appreciated clinically. Against this hypothesis is the observation that the lesions in this case were not located exclusively in areas most susceptible to this sort of injury. Although hypoxia remains a possibility, it may be that the lesions resulted from extensive infection of cortical neurons with resultant necrosis and cystic changes. Some support for this concept is provided by the finding of abundant inclusion bodies in these necrotic foci. This type of lesion has also been seen in experimental measles encephalitis in the hamster.[40]

The use of ACTH or corticosteroids in both these patients introduces a concern that may be pertinent to the consideration of these pathologic findings. Whether steroids in high dosages and for the durations cited could exert an immunosuppressive effect and lead to enhanced neuroviricidal changes is a question impossible to answer. It seems unlikely, however, since neither patient was immunologically impaired in any detectable clinical sense and despite profound debility, neither patient experienced a significant problem with sepsis.

The fact that both these patients received attenuated live measles vaccine (with gamma globulin) should be singled out for comment. The status of both patients' immunity to measles was uncertain at the time of vaccination. One patient (Case 2) had a known exposure years before and may well have had a subclinical infection leading to permanent immunity. Even assuming that both patients' initial infection with this virus followed vaccination, the cumulative experience to date with this disease stresses its occurrence in individuals after naturally occurring measles. There have been a few documented cases occurring after measles immunization,[23,41] but their infrequency, despite the millions of vaccinations administered in the past 7 years, is reassuring evidence against there being any significant causal relationship. On the other hand, it might be argued that since the vaccine strain of virus is attenuated and of low neurovirulence,[42] it might be expected to lead to a significant reduction in the incidence of SSPE in the years ahead. Obviously, more data is required to advance our understanding of what is now only conjecture.

The virus recovered from the brain of Case 2 has been identified as the measles agent by the standard prescribed technics. It is in-

teresting that it remained inapparent in cultures for such an extended period before producing cytopathology and demonstrable infectivity. Even though others have had difficulty retrieving infectious virus until later passage, giant cells and syncytia appeared early. The significance of this, if any, is unclear at present except to stress the importance of persistence in maintaining cultures despite early signs of negativity.

The virus was isolated only from the left frontal region, an area where inclusion bodies were most abundant. This serves to emphasize that biopsy of one region of brain may well be negative for virus at a time when the process is active in adjacent regions. It further underscores the importance of early postmortem culture of brain tissue from multiple sites as an approach to future studies of this and related diseases.

Summary

Clinical and postmortem studies of 2 patients with uncommon manifestations of subacute sclerosing panencephalitis (SSPE) are described. Lesions were widely distributed throughout the nervous system, including the eye, brainstem, cerebellum, and spinal cord. Laminar necrosis was particularly prominent in one case and evident microscopically in the other. Electron microscopy of the brain after death disclosed, in each case, filamentous structures characteristic of SSPE. Both patients had high titers of measles virus antibody in the CSF and, in one case, the measles virus was recovered from cerebral tissue obtained at autopsy.

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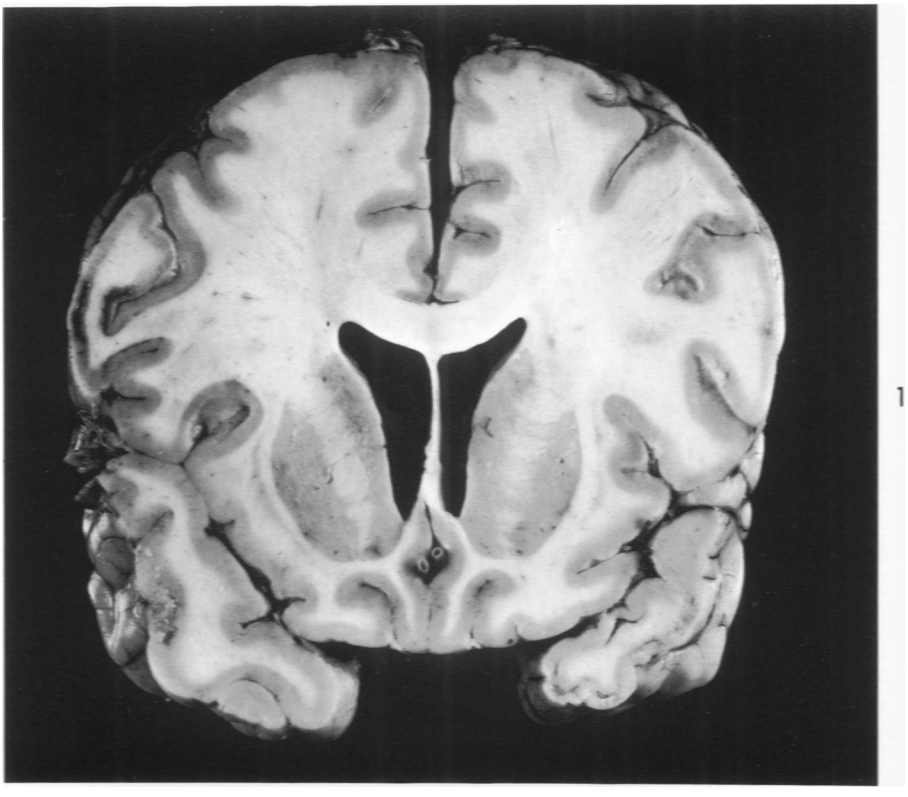
The authors wish to acknowledge the technical assistance of Mr. C. Bishop and Mr. B. Lloyd, and the prosectors of the autopsies: Dr. J. Hall (Case 1) and Dr. C. Daniels (Case 2).

Presented in part at the annual meeting of the American Association of Neuropathologists, Atlantic City, New Jersey, June 12 to 14, 1970.



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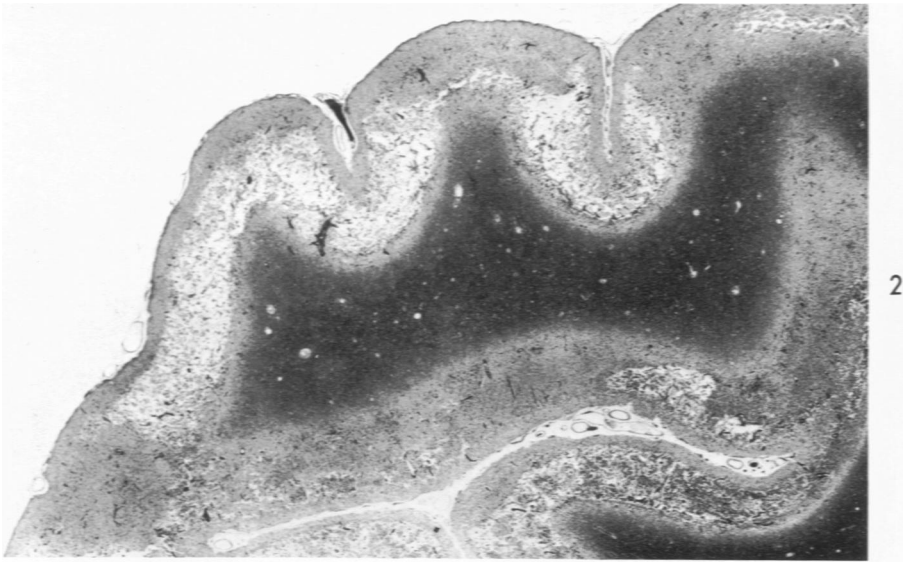

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Fig 1.—Coronal section of the brain of Case 2 demonstrates cortical laminar necrosis, especially prominent in left temporal cortex. Mild cerebral edema and focal cortical hemorrhages are also evident.

Fig 2.—Extent of laminar cortical necrosis in Case 2 is shown under higher magnification. H&E with luxol fast blue. × 3.

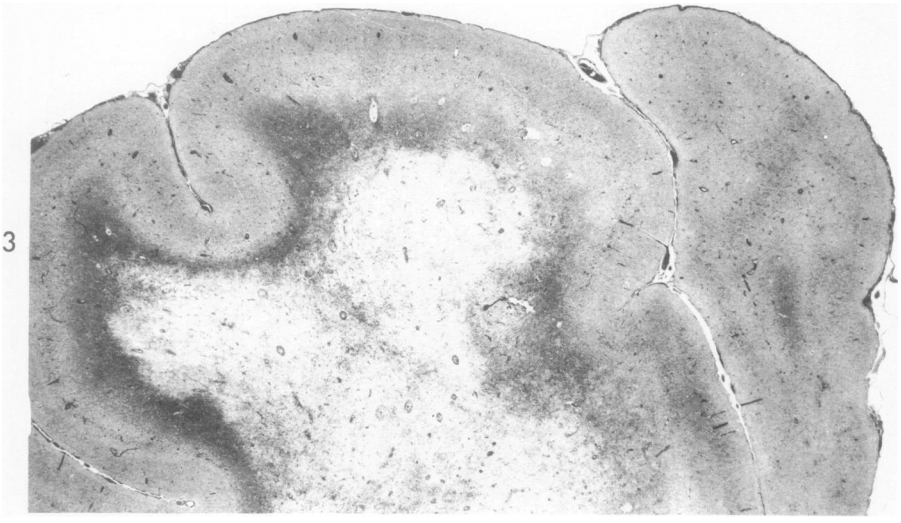

Fig 3.—Marked demyelination of cerebral white matter from occipital lobe in Case 2 is shown. H&E with luxol fast blue. × 3.

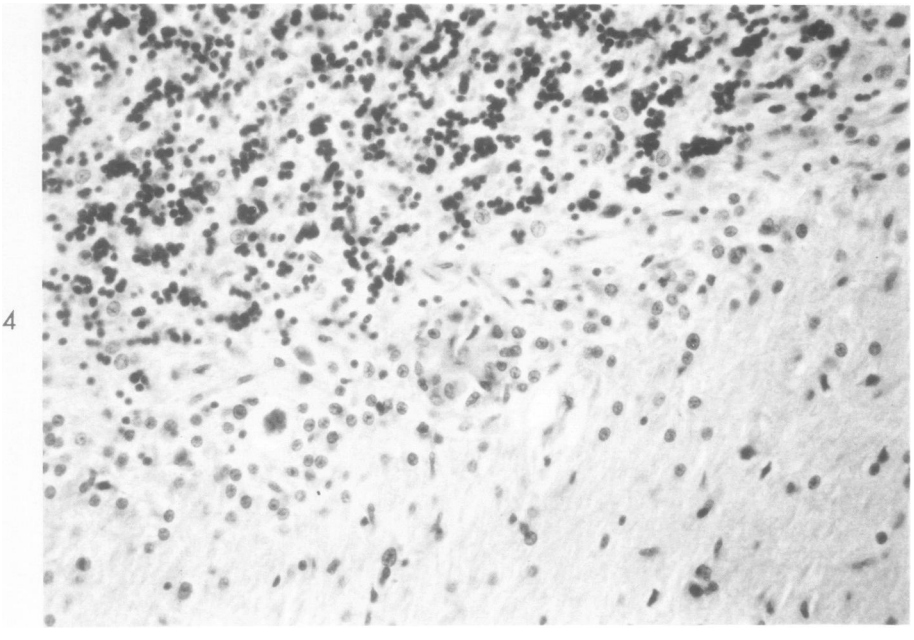

Fig 4.—Diffuse loss of Purkinje cells, prominent proliferation of Bergmann's glia, and glial nodule in cerebellar cortex. H&E with luxol fast blue. × 680.

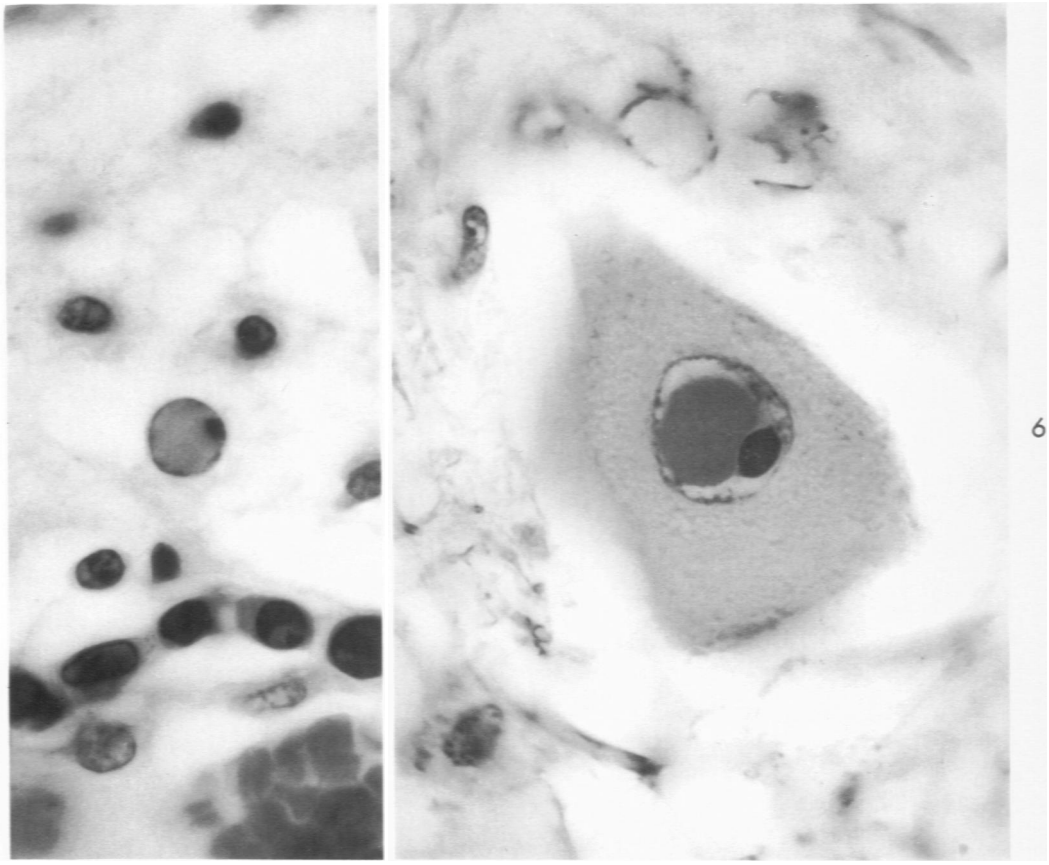

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Fig 5.—Most frequently encountered inclusion bodies in both cases were small, eosinophilic intranuclear structures with marginated chromatin. These were found in astrocytes, oligodendroglia, and occasional neurons throughout nervous system. H&E with luxol fast blue. × 1200.

Fig 6.—Many anterior horn cells of lumbar spinal cord of Case 1 contained eosinophilic intranuclear inclusion bodies, with clear halo between inclusion and peripheral chromatin (Cowdry Type A). This type of inclusion body was also observed in other parts of nervous system, particularly in Case 1. H&E with luxol fast blue. × 1200.

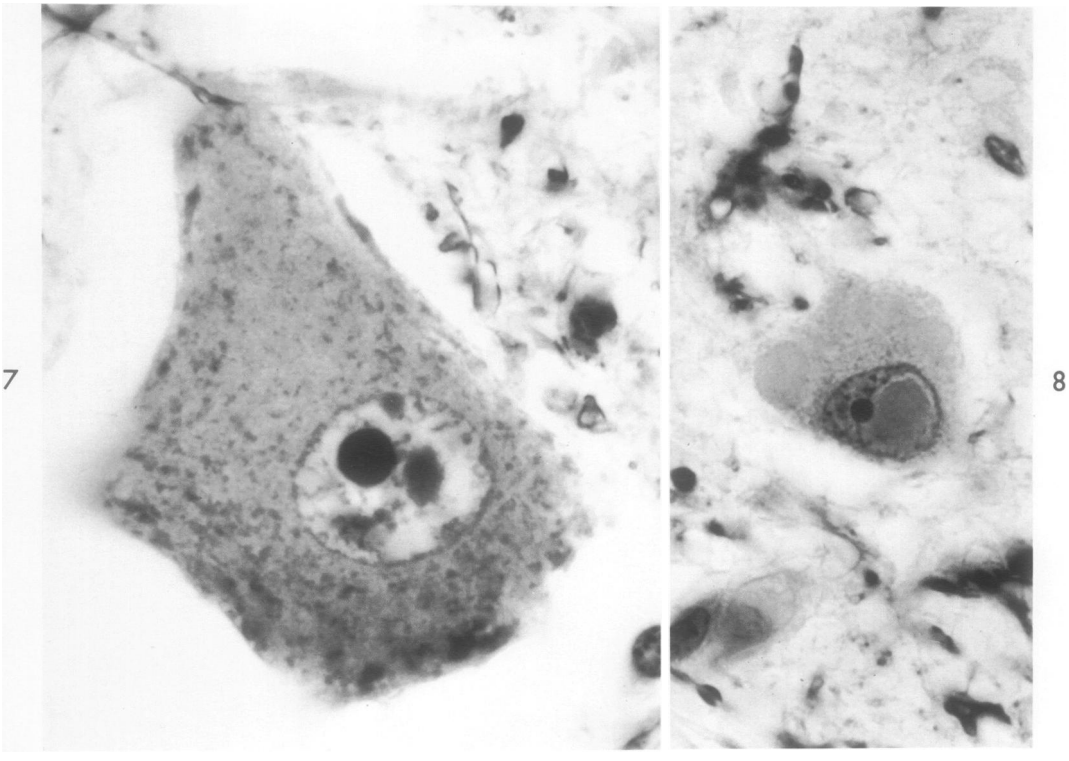
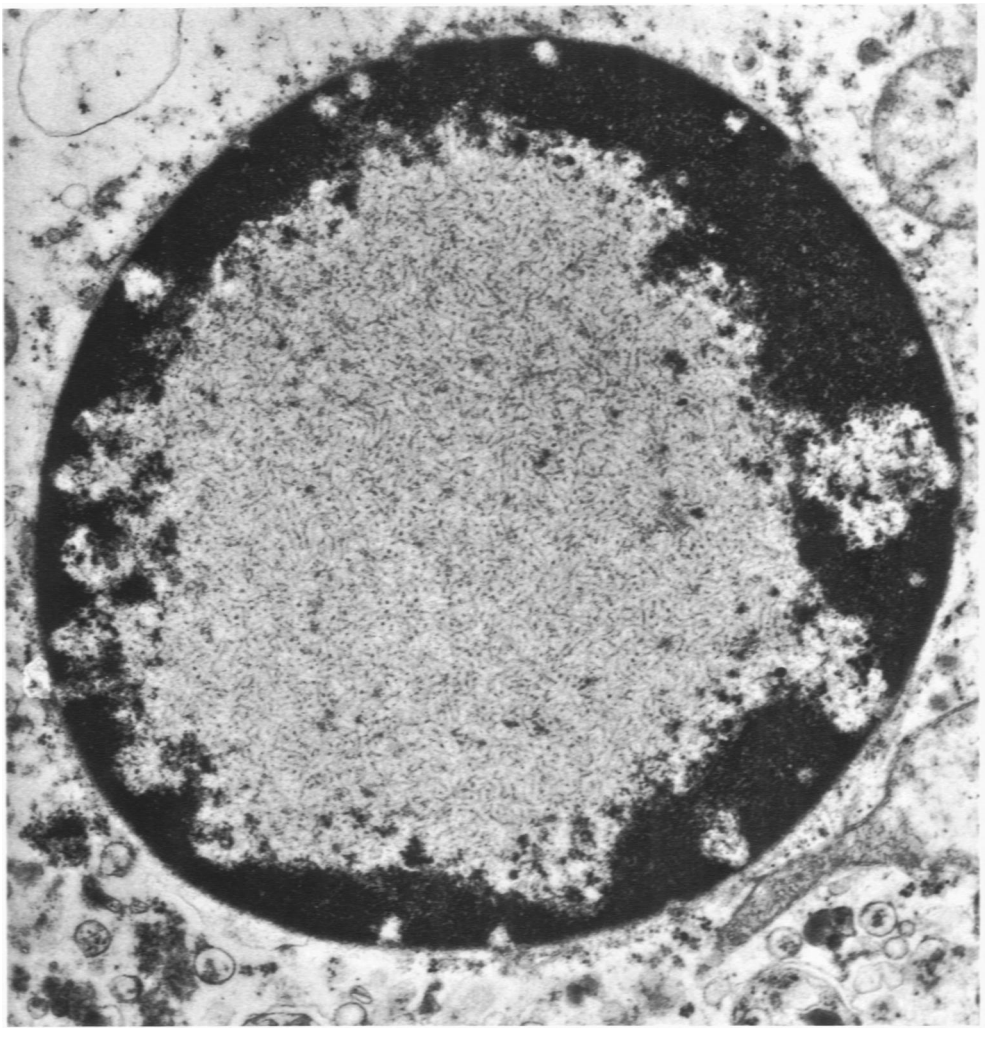


Fig 7.—Small, eosinophilic, intranuclear inclusions, known as Cowdry Type B, were occasionally seen in neurons from both cases. This figure illustrates inclusion in anterior horn cell from lumbar spinal cord of Case 1. H&E with luxol fast blue. × 1200.

Fig 8.—Eosinophilic cytoplasmic inclusions were also found in some neurons. H&E with luxol fast blue. × 1200.

Fig 9.—In both patients, electron microscopy revealed multitubular structures measuring 150–260 Å in diameter in nuclei of neurons and neuroglia. × 13,000.

Fig 10.—Tubular filamentous particles morphologically indistinguishable from those within nuclei were occasionally observed in cytoplasm with electron microscope. × 30,000.



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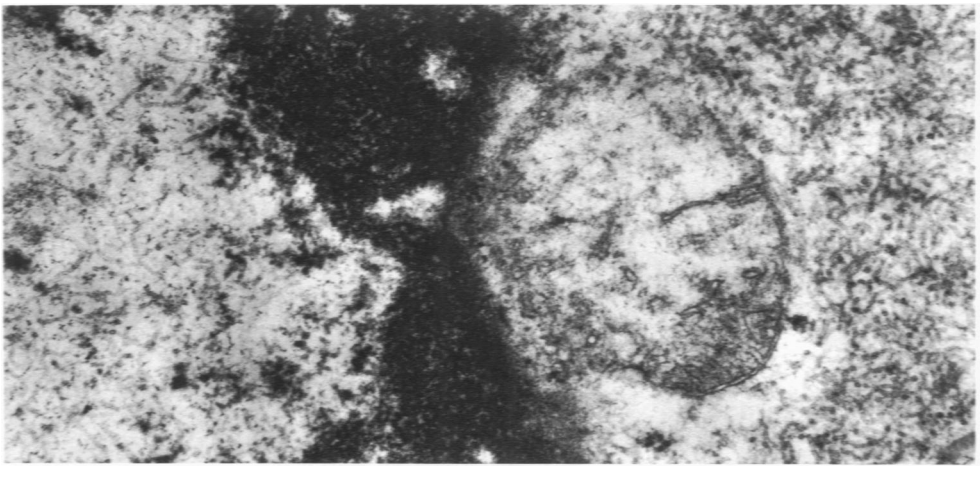

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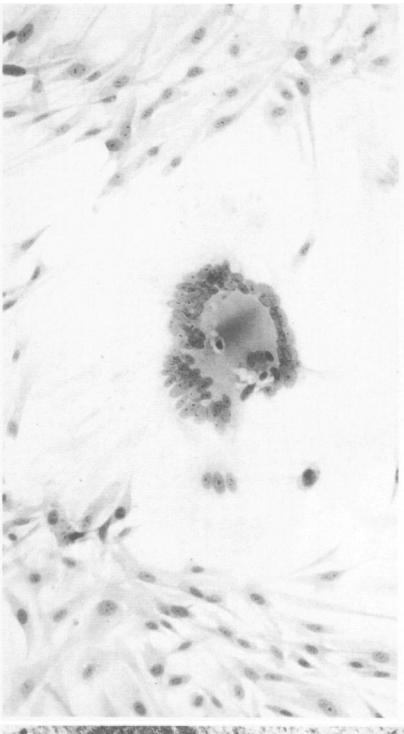

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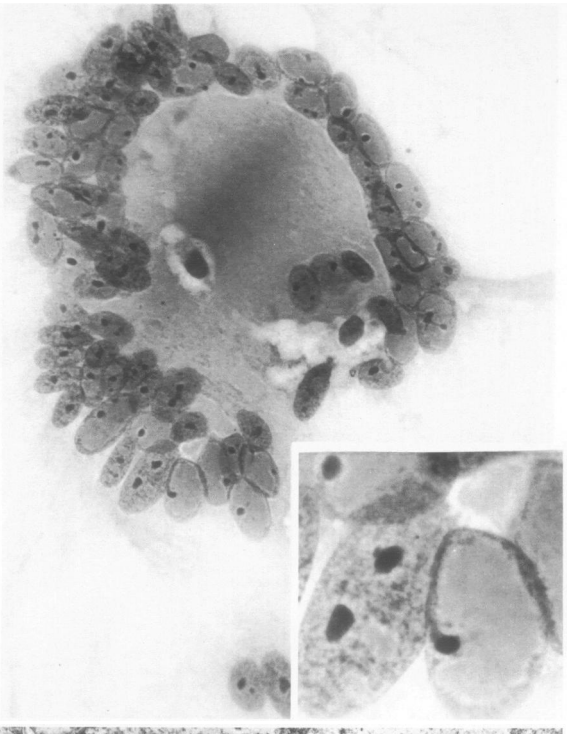

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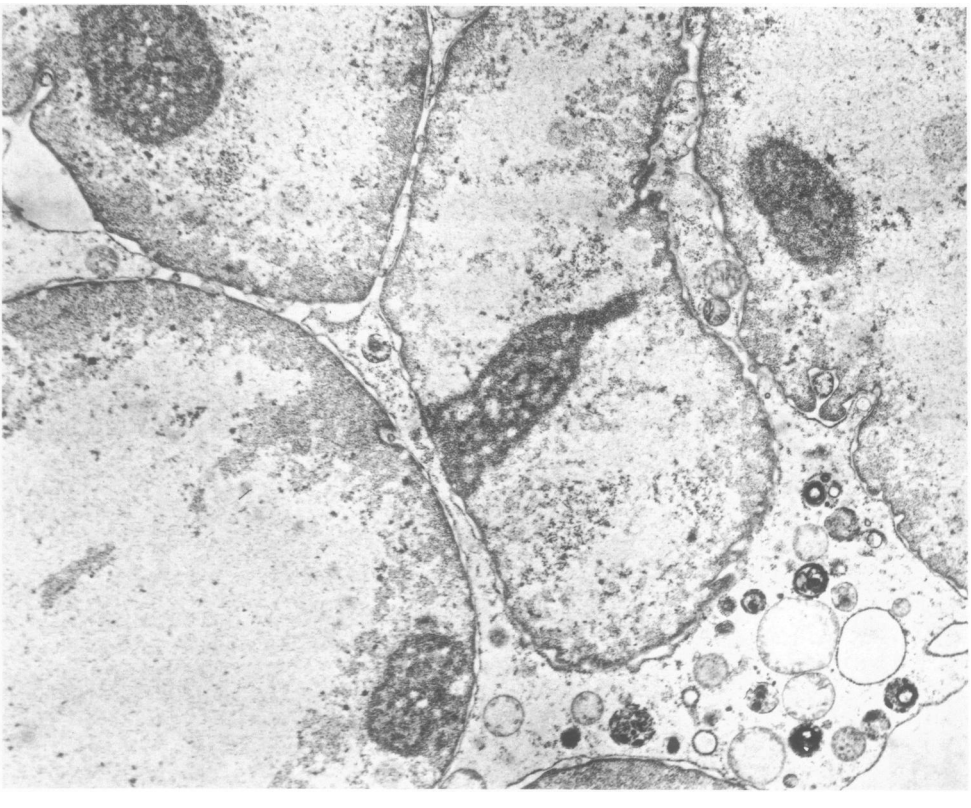
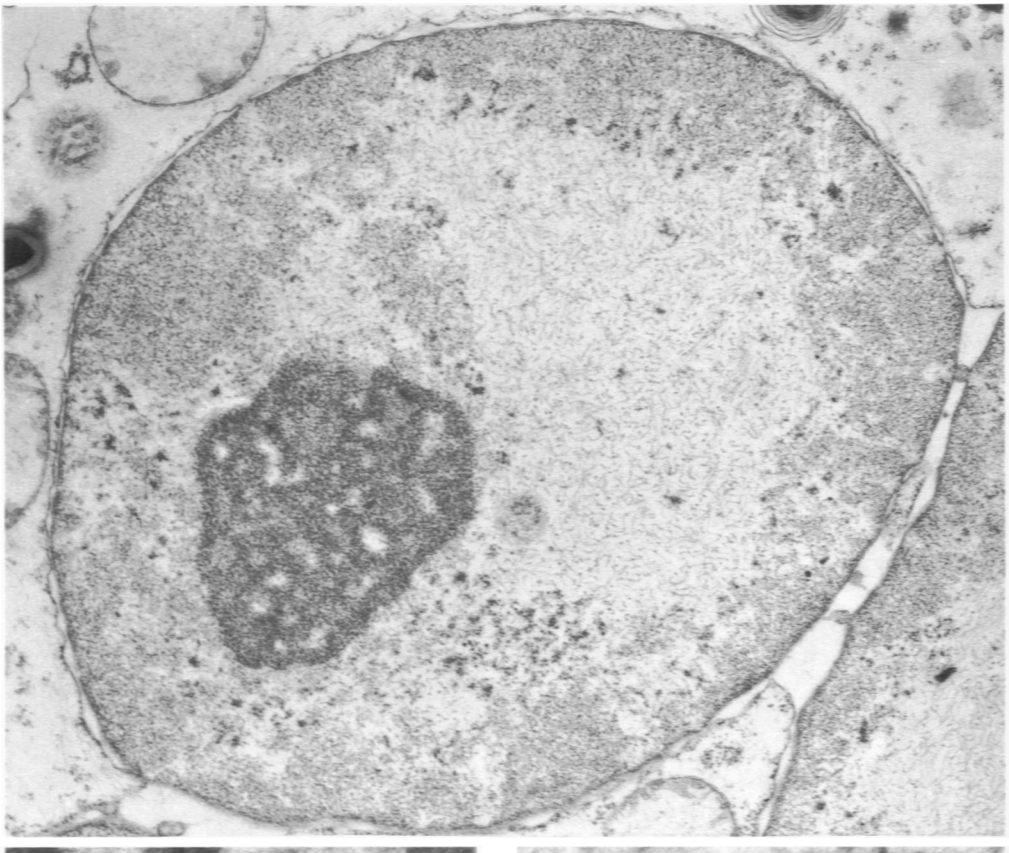


Fig 11A.—This postmortem cell culture of the brain shows a syncytium surrounded by stellate fibroblast-like cells (eighth passage, 3 months after primary inoculation). H&E. × 150. **B.**—Under higher magnification intranuclear (Cowdry Type A) and intracytoplasmic inclusions are evident in syncytial giant cell. H&E. × 400. Inset, × 1200. **Fig 12.**—Nuclei of syncytial giant cell in brain culture contain aggregates of tubular filamentous structures, which displace chromatin to the periphery of each nucleus. × 5,600.



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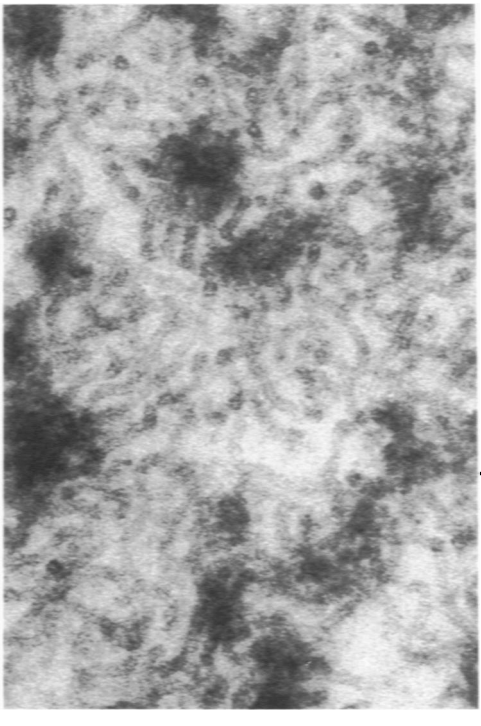

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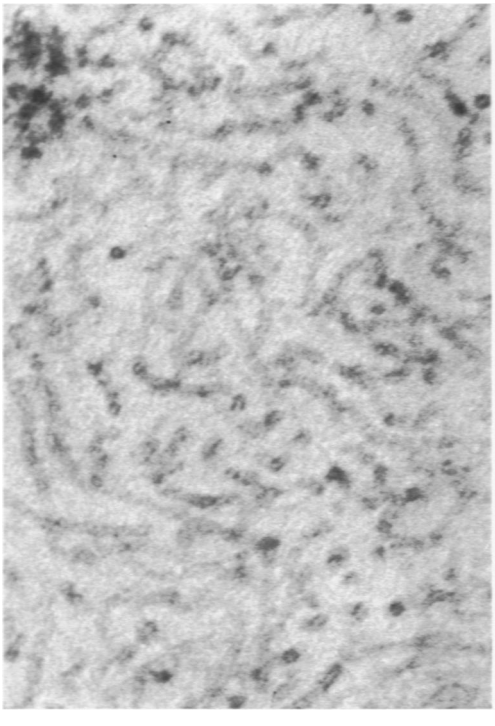

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Fig 13.—Electron microscopy of part of cell culture illustrated in Fig 11 reveals same microtubular structures within nucleus that were seen in postmortem material from brain of both cases. × 12,000. **Fig 14.**—These particles were present in nucleus of astrocyte in the postmortem material from Case 1. × 100,000. **Fig 15.**—Morphologically identical particles were seen in nucleus of tissue culture cell (Case 2). × 100,000.

[*End of Article*]