Cucumber Shaped and 35 nm Particles in Creutzfeldt-Jakob Disease

DIKRAN S. HOROUPIAN AND R. T. ROSS

SUMMARY: *A 63 year old female with the ataxic form of Creutzfeldt-Jakob disease (CJD) is presented. In addition to amyloid plaques which were not associated with Alzheimer's neurofibrillary tangles, rare profiles similar to those reported in Scrapie were also seen. To our knowledge, these profiles have never been observed in CJD and their presence in this condition adds a further morphologic similarity between the human and animal forms of subacute spongiform "viral" encephalopathies.*

RÉSUMÉ: *Une femme de 63 ans avec une forme ataxique de la maladie de Creutzfeldt-Jakob (CJD) est présentée. En plus de plaques amyloides qui n'étaient pas associées aux formations neurofibrillaries d'Alzheimer, de rares profils semblables à ceux rapportés dans le Scrapie étaient également observés. A notre connaissance, ces profils n'ont jamais été observés dans le CJD et leur présence, dans cette condition, ajoute une nouvelle similarité morphologique entre les formes humaines et animales d'encéphalopathies "virales" spongiformes subaigues.*

From the Departments of Pathology and Medicine, Health Sciences Center and Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

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Reprint requests to Dr. D. S. Horoupian, Department of Pathology, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, Michigan 48202 U.S.A.

INTRODUCTION

Creutzfeldt-Jakob disease (CJD), Kuru and Scrapie are almost indistinguishable histologically. PAS positive plaques once considered characteristic of Kuru were later observed in CJD (Horoupian et al, 1972) and in mice inoculated with special Scrapie strains (Frazer and Bruce, 1973), but the cucumber-shaped (Narang, 1974) and the 35 nm particles (Bignami and Parry, 1972) have only been described in natural and experimental Scrapie. We wish to report in this paper, the finding of similar particles in the brain biopsy of a 63 year old female with the ataxic form of CJD.

Case History:

The patient, born in 1909, was well until May 1972. She then experienced dizziness, "loss of balance" and veering to the left when walking. She was admitted to the Health Sciences Center, Winnipeg in September 1972. Plain x-ray of the skull, myelogram, brain scan, audiogram, VDRL and other laboratory investigations were all negative. She had had no major illness in the past. During the following three months, she gradually developed pronounced mental deterioration, stuttering and ataxia with choreiform movements but no myoclonus. Diffuse, abnormal slow-wave activity appeared in the EEG. Serum alkaline phosphatase was elevated — 168 U/L, but the liver biopsy was not remarkable (Roos et al, 1973). Isotope encephalogram and pneumoencephalogram were negative. On January 15, 1973, a brain biopsy was performed through a right frontal burr hole. At operation, she was noted to have marked cortical atrophy and a profuse amount of cerebrospinal fluid in the cranial subarachnoid space. The brain biopsy (S784) was diagnostic of subacute spongiform viral encephalopathy. Following the operation, her condition deteriorated, she spiked a temperature and was difficult to arouse. Her plantar responses were extensor and her extremities were held in flexion and were rigid. She expired a month later.

Material and Methods:

A portion of the brain biopsy and specimens from the unfixed cerebellum and liver obtained two hours after death were stored in liquid nitrogen and sent to Dr. D. C. Gajdusek's laboratory in Bethesda, Maryland. These were inoculated into capuchin monkeys. The monkeys were symptomless 24 months later (Dr. R. D. Traub, Laboratory of Central Nervous System Studies, Health, Education and Welfare Department. NIH. Bethesda).

The brain biopsy and representative specimens obtained from the fresh brain, cerebellum and liver were processed for electron microscopy.

General Autopsy Findings (73B35)

The deceased was severely emaciated and weighed 27 kilograms. There were patchy areas of pulmonary fibrosis and emphysema. Minimal fatty changes and inspissated eosinophilic material were present in the liver and pancreatic ductules respectively.

Gross Neuropathological Findings:

The unfixed brain weighed 1.040 gm. and displayed generalized atrophy. The cerebellum was relatively more affected than the cerebrum. There were bilateral minimal

subdural hematomata. The vessels were smooth and devoid of obvious atheroma. Coronal sections displayed equivocal attenuation of the cortical ribbon. The ventricles were dilated and the head of the caudate nucleus was flat and atrophic. The putamen, fornix and medial thalamic nuclei were dusky. The thalami appeared slightly spongy.

Folial atrophy was more marked in the vermis, flocculus and tonsils. Also, the folia of the superior surface of the cerebellar hemispheres were thinner than those of the inferior surface.

Histological Findings:

The changes in the biopsy were essentially the same as those seen in the autopsy material with the exception that the latter were more pronounced. These changes consisted of sponginess of neuropil, astrocytosis and neuronal loss. A few argentophilic plaques were present in the cerebral cortex, but the plaques were only faintly congophilic. Alzheimer's neurofibrillary tangles could not be identified with certainty even in the pyramidal layer of Ammon's horn. These changes were present diffusely in the frontal parietal and occipital lobes, but were rather patchy in the temporal lobes. Vacuoles were more common in the deeper layers, especially layer four. Sommer's sectors and subiculum complex also displayed vacuolation. Astrocytosis, although present with variable intensity in the cortex was most pronounced in the basal ganglia, especially the caudate nucleus, where neuronal loss was very extensive. The thalami were also severely involved, especially the dorsomedial nuclei and the massa intermedia. The substantia nigra and locus ceruleus showed focal neuronal loss, astrocytosis and excess extracellular neuromelanin. The pontine neurons were shrunken and had pyknotic nuclei and intensively eosinophilic cytoplasm. Occasional cytoplasmic vacuolation was present. "Drop-out" of neurons were also observed in the inferior olivary nuclei with marked increase in the lipochrome content of the surviving cells. Intense granular cell degeneration, astrocytosis, empty baskets, patchy loss of Purkinje's cells and "torpedoes" were present in the cerebellum.

Electron Microscopic Study of the Biopsy Material:

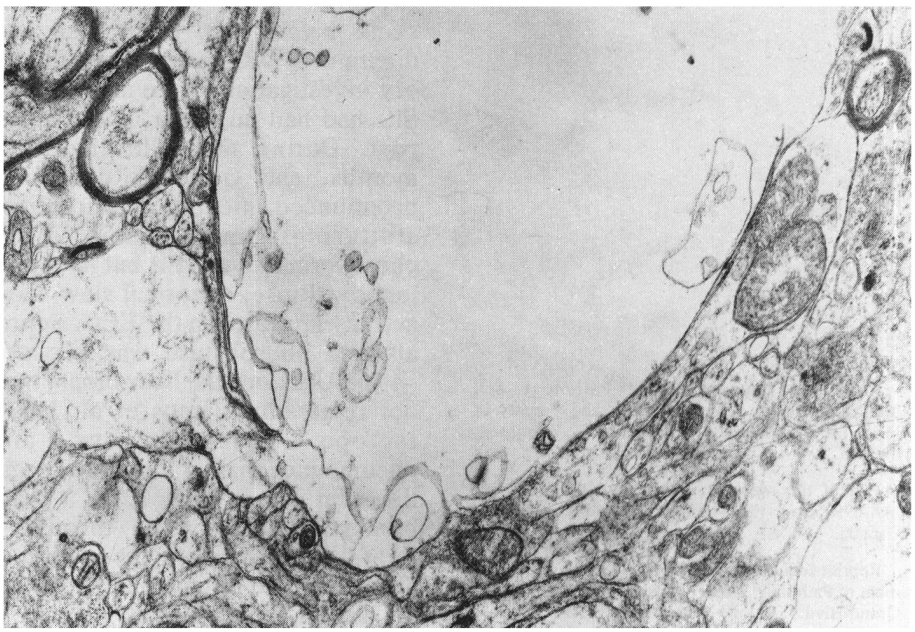
This revealed large as well as smaller vacuoles. They were membrane bound and some of the larger vacuoles contained membranous coils similar to those described by Lampert et al. (1972). They were mostly present in astrocytes or their processes and occasionally in neurons. The smaller vacuoles were mainly in dendrites or in cell processes so ballooned that their origin was obscured (Fig. 1). A few amyloid plaques, partially surrounded by degenerating neurites were also observed (Fig. 2). These neuronal cell processes were distended with a variety of dense bodies, distorted mitochondria, electron dense and lucent vesicles of varying sizes and tubulo-vesicular networks (Fig. 3). There were no twisted tubules of Alzheimer's neurofibrillary tangles. On one occasion, a hematogenous cell was observed to contain membrane bound, stacks of filaments that had the periodicity of amyloid. (Fig. 4).

Rarely, two kinds of abnormal profiles, morphologically dissimilar, were encountered. One of them was formed of spherical or oblong particles measuring on the average, 32.5 x 16.5 nm and had an electron lucent central core (Fig. 5). They seemed to belong to an intricate tubular network that filled up the cell processes in which they existed. The second kind consisted of aggregates of spherical bodies, some of which were dense, but by-and-large they were mostly vesicular. The mean diameter was 35 nm and were mostly present in villous-like projections in cell processes, the origin of which was difficult to identify (? boutons terminaux) (Fig. 6). The possibility that these cell processes belonged to "dark" cells, as these were occasionally seen, could not be excluded with certainty. However, on one occasion, they were more evenly distributed in a cell process that did not display vacuolation or villous-like formations (Fig. 7). Occasionally, astrocytes having hydropic perikarya contained large, irregular, lipid inclusions.

DISCUSSION

The patient's initial symptoms were those of cerebellar dysfunction

Figure 1—Bullous vacuolization of a neurite that contains membranous coils (X 12.000).



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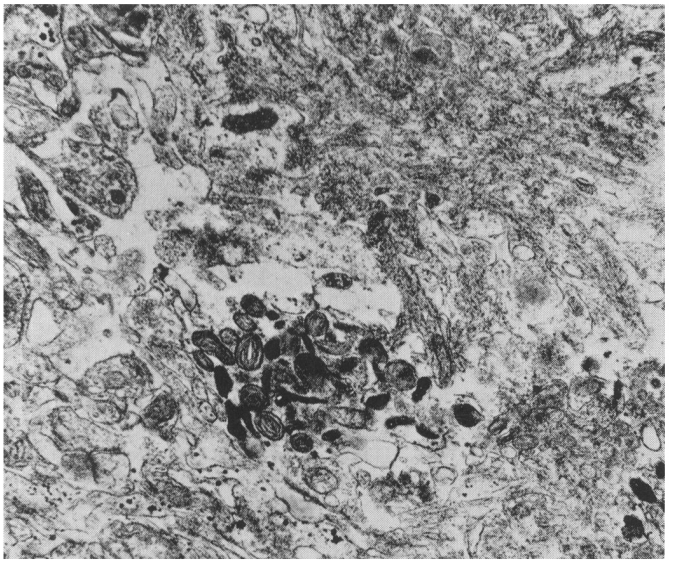


Figure 2—Stellate amyloid plaque from cortical biopsy, surrounded with occasional degenerating neurites (X 16.150).

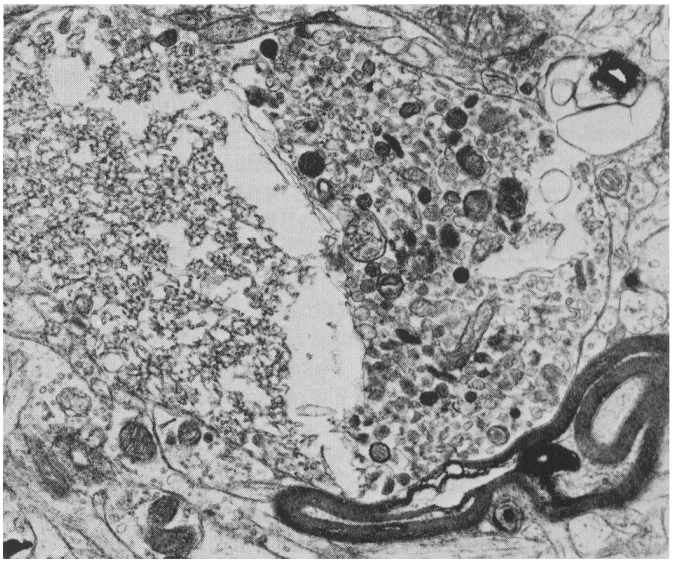


Figure 3—A neuronal cell process distended with dense bodies and altered mitochondria in apposition with a neurite that contains tubulovesicular network (X 15.500).

which later were complicated by dementia and choreiform movements. The pathological findings consisted of status spongiosus, astrocytosis and neuronal loss which were more pronounced in the basal ganglia, thalami and cerebellum. The sequence of neurological manifestations and the pathologic findings were therefore consistent with those described in the ataxic variant of Creutzfeldt-Jakob disease (Gomori et al, 1973). In addition to these findings, argyrophilic plaques which in EM studies consisted of stellate amyloid bundles were present in the cortex. Similar to the two cases reported by Hirano (1972) twisted tubules of Alzheimer's neurofibrillary tangles (Wisniewsky et al, 1970) were absent. Membrane bound amyloid fibrils were also seen in a hematogenous cell. The source of amyloid in these cells has been a matter of controversy. Terry et al (1964) considered it as locally produced by these cells and have based their contention on the observation made by Heefner and Sorenson (1962) that amyloid bundles were present in the cytoplasm of splenic reticulo-endothelial cells and also, on the fact that in their cases the intracellular bundles were not surrounded by membrane. In the present case, the intracellular amyloid

Figure 4—A hematogenous cell displaying membrane bound stacks of amyloid fibrils (X 9.500).

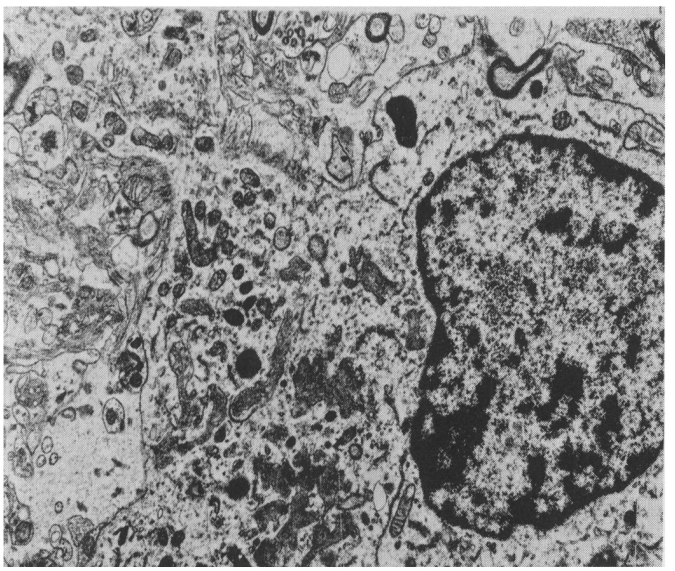
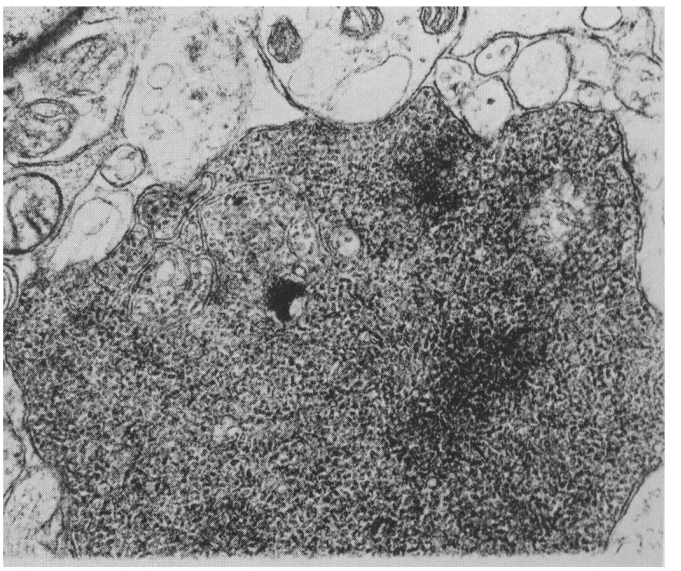


Figure 5—Cucumber-shaped particles in ? oligodendroglial cytoplasm (X 42.000).



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was membrane bound and whether this indicates phagocytosis or not remains conjectural.

Two profiles, hitherto not reported in CJD were encountered. They were altogether very rare. One type was very similar to the cucumber-shaped particles described by Narang et al (1972) in Scrapie and were present in what was interpreted as oligodendroglial cytoplasm. Field and Narang (1972) have also occasionally observed them in the oligodendroglia of experimental rat Scrapie. They seemed to be part of a tubular network that was distinctly different from the abnormal arrangement of endoplasmic reticulum that has been described in viral or suspected viral diseases (Horoupian et al, 1974). Interestingly, similar profiles were also recently reported in an unusual case of amyotrophic lateral sclerosis that followed recurrent lymphocytic meningitis (Norris et al, 1975). This is of special interest since amyotrophic lateral sclerosis may be one of the abiotrophies, the etiology of which may be a slow virus (Gibbs and Gajdusek, 1972). The second kind of abnormal profiles were spherical and were mainly observed in villous-like formations. They resemble the 35 nm particles that were reported by Bignami and Parry, 1972 in natural Scrapie, while those that were more evenly distributed were mostly vesicular and were similar to the spherical bodies described by Lampert et al (1971) in experimental Creutzfeldt-Jakob disease in chimpanzees.

A number of different particles have been described in Scrapie (David-Ferreira et al, 1968) and a few have been postulated to be related in some ways to the transmissible agent of Scrapie, but as yet no definite proof exists, to support any of these contentions. Whatever the significance of these particles may be, their presence in a case of CJD is intriguing and adds a further morphologic similarity between the human and animal forms of subacute spongiform "viral" encephalopathies.

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Figure 6—35 nm particles in villous-like projections in a cell process (X 13.500).

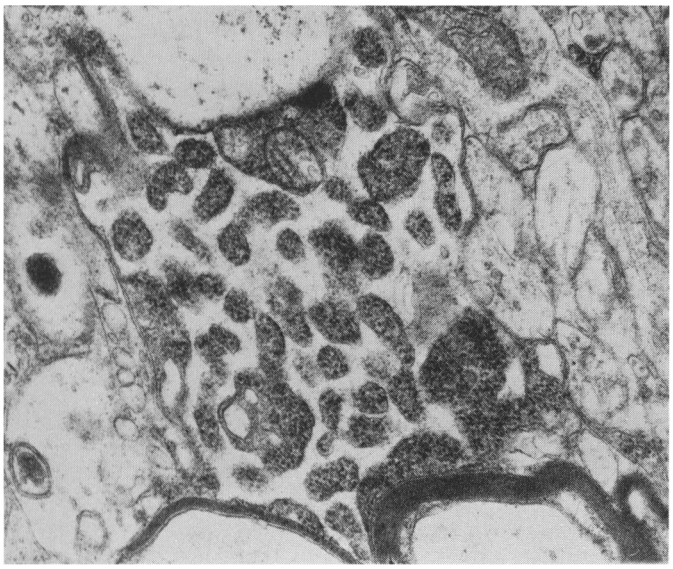
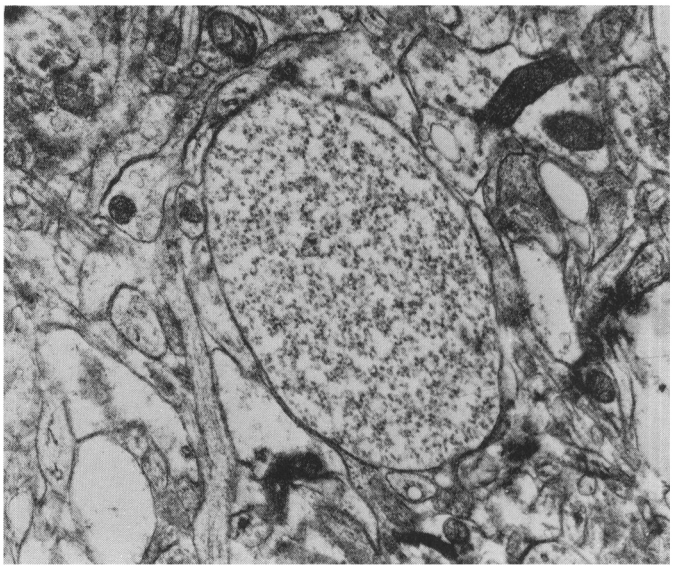


Figure 7—Cell process filled with spherical and ovoid profiles (X 14.400).



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